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DEVELOPMENT AND CHARACTERISATION OF NON-IONIC SURAFACTANT VESICLES (NIOSOMES) FOR OCULAR DELIVERY OF DICLOFENAC SODIUM

Dissertation submitted to

The TamilNadu Dr. M.G.R. Medical University, Chennai,

In partial fulfillment of the requirement for the award of the degree of

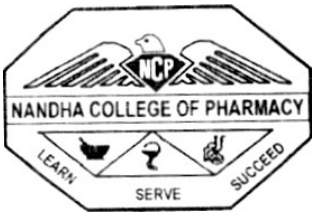
MASTER OF PHARMACY (PHARMACEUTICS)

Submitted by

SANDEEP. S

Under the guidance of

Prof. D. KARTHIKEYAN, **M. Pharm., (Ph.D.,)**



NANDHA COLLEGE OF PHARMACY

ERODE – 638 052 (TAMILNADU)

MARCH 2008

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled, **"DEVELOPMENT AND CHARACTERISATION OF NON-IONIC SURFACTANT VESICLES (NIOSOMES) FOR OCULAR DELIVERY OF DICLOFENAC SODIUM"** submitted to The TamilNadu Dr. M.G.R. Medical University, Chennai, was carried out by **Mr. SANDEEP. S** Department of Pharmaceutics, Nandha College of Pharmacy, Erode-52 for the partial fulfillment for the award of degree of Master of Pharmacy in Pharmaceutics under my supervision.

This work is original and has not been submitted in part or full for any other degree or diploma of this or any other university.

Date :

Place : Erode

DECLARATION

The work presented in this thesis entitled **"DEVELOPMENT AND CHARACTERISATION OF NON-IONIC SURFACTANT VESICLES (NIOSOMES) FOR OCULAR DELIVERY OF DICLOFENAC SODIUM"** was carried out by me in the Department of Pharmaceutics, Nandha College of Pharmacy, Erode-52 under the direct supervision of **Prof. D. Karthikeyan, M.Pharm., (PhD).,** Vice Principal and H.O.D., Dept. of Pharmaceutics, Nandha College of Pharmacy, Erode-52

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other University.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

µg	-	Microgram
µm	-	Micrometer
Abs	-	Absorbance
cm	-	Centimeter
CPP	-	Critical Packing Parameter
Cum%	-	Cumulative Percentage
Fig.	-	Figure
gm	-	Gram
HLB	-	Hydrophilic Lipophilic Balance
HPLC	-	High Performance Liquid Chromatography
Hrs.	-	Hours
Luv	-	Large Unilamellar Vesicle
mg	-	Milligram
Min.	-	Minutes
MLV	-	Multi Lamellar Vesicle
nm	-	Nanometer
Sqrt	-	Square root
SUV	-	Small Unilamellar Vesicle

INTRODUCTION

Development of new drugs is difficult, expensive and rather time consuming, as it involves the processes like preclinical testing, investigational new drug application (IND), clinical trials, phase I, II, & III, new drug application (NDA) and FDA approval. Improving safety and efficacy of existing drugs is being attempted by using different methods such as individualizing drug therapy, dose titration and therapeutic drug monitoring and, most importantly, delivering drugs at controlled rates at targeted sites. Drug delivery systems could provide extended circulating half-lives so that less drug is required for therapeutic effectiveness relieving the patient from side effects caused by non-specific tissue uptake and provide protection against enzymatic degradation.

Today, lipid and nonionic surfactant based drug delivery systems have drawn much attention from researchers as potential carriers of various bioactive molecules that could be used for therapeutic applications. Several commercial liposome/niosome-based drugs have already been marketed with a great success. For example, liposomes and niosomes have been used to encapsulate Colchicines,[1] Tretinoin,[2,3] Dithranol,[4] Enoxacin,[5] Estradiol,[6] Methotrexate[7] for applications such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccines. [8-15]

Liposomes have been reported to increase drug stability, enhance therapeutic effects, prolong circulation time and promote uptake of the entrapped drugs into target site while drug toxicity is diminished. However, there are problems in the general applications of liposomes. In an aqueous system, liposomes have problems regarding degradation by hydrolysis of phospholipid molecules. Problems with the physical and chemical stabilities of aqueous suspensions of liposomes have been addressed by many researchers, who introduced a dry free-flowing granular product that could be immediately hydrated before use. One alternative of phospholipids is the hydrated mixture of cholesterol and non-ionic surfactants such as alkyl ethers, alkyl esters or alkyl amides non-ionic surfactants. This type of vesicle formed from the above mixture has

been known as niosomes or non-ionic surfactant vesicles.[16] Here we are concerned about the ocular delivery of the drug by vesicular system.

1. Ophthalmic drug delivery[16]

An ophthalmologist prefer topical application to the eye as a method of treating eye diseases since systemic involvement is usually, but not always minimal. For some drugs such as anti-cholinesterase and most cholinergic drug, treatment of the eye by systemic route would be impossible because of their toxicity. Most of the current ophthalmic preparations are available as sterile buffered isotonic solutions, because a majority of ophthalmic drugs are water soluble. In fact solution dosage forms are preferred, as drops are easier to administer. However in situation where there is solubility limitation, or when a prolonged action is desired, disperse system such as suspension, gelled system and ointment are indicated

The unique anatomy, physiology and biochemistry of eye tenders this organ exquisitely impervious to foreign substance, thus presenting a constant challenge to the formulator to circumvent the protective barrier of the eye without causing any permanent tissue damage. Currently the knowledge in this field is rapidly expanding and many concepts and drug delivery strategies are emerging out.

1.1. Eye: Anatomical and physiological overview [17]

The human eye has spherical shape with 23 mm diameter. The structural components of the ball are divided in to three layers.

1. The outer most coat of clear transparent cornea and white opaque sclera
2. The middle layer comprises the iris anteriorly, the choroids posteriorly and ciliary body as intermediate part; and
3. The inner layer is the retina which is an extension of the central nervous system.

The fluid systems in the eye, aqueous humor and vitreous humor also plays an important role in maintaining ocular pressure. Cornea is optically transparent tissue that acts as the principle refractive element of the eye. The corneal diameter is about 11.7 mm with a radius of curvature (anterior surface) about 7.8 mm. The corneal thickness is 0.5-0.7 mm and it is thicker in the center. The cornea is composed of epithelium, bowmen's membrane, strauma, descement's membrane and endothelium. The relative thickness of corneal epithelium (50-90mm), strauma and endothelium are about 0.1, 1.0 & 0.01 respectively. The shape of cornea and lens adjusted by ciliary body. A mesh of blood vessels, the choroids supplies oxygen and glucose to the retina. Lachrymal gland secrets tear that wash foreign bodies out of and keep the cornea from drying out. Blinking compresses the lachrymal sac and allows the lachrymal fluid to move out which moistures the eye surface. The drugs in ophthalmic preparation reach inside the eye through cornea, because the structure of cornea consists of epithelium–strauma–endothelium, which is equivalent to a fat-water-fat system. The penetration of non polar compound through the cornea depends on their oil/water partition coefficient.

The blood ocular barrier normally keeps most drugs out of eye. However inflammation breaks down this barrier allowing drug and large molecule to penetrate in to the eye. As the inflammation subsides the barrier will return.

The blood barrier comprises the following sites.

1. The aqueous humor blood barrier between the ciliary epithelium and capillaries of iris
2. Blood retinal barrier: Non-fenestrated capillaries of the retinal circulation and tight junction between retinal epithelial cell preventing passages of the large molecule from choroid capillaries of retina.

1.2. Absorption of drug in eye [18-20]

It is often assumed that drugs administered in to the eye are totally and rapidly absorbed however these are few factors which affect the drug delivery to eye. Absorption of drug takes place in corneal or non corneal route. The non corneal route involves absorption across the sclera and conjunctiva which restrains the entry of drug into aqueous humor. Maximum absorption takes place in cornea which leads drug to aqueous humor. A big portion of the drug that administered to the eye was lost leading to poor ocular bioavailability.

1.2(a) Drug elimination from lachrymal fluid

Drugs are mainly eliminated from the precorneal lachrymal fluid by solution drainage, lacrimation and non productive absorption by conjunctiva of the eye. Only little percentage of applied doses delivered in to intra ocular tissue, while the major part (50-100%) of dose is absorbed systemically. The pre-corneal constraints include:

1. Dilution of drug by over flow
2. Dilution of drug by tear turn over

3. Nasolachrymal drainage conjunctival absorption
4. Enzyme metabolism

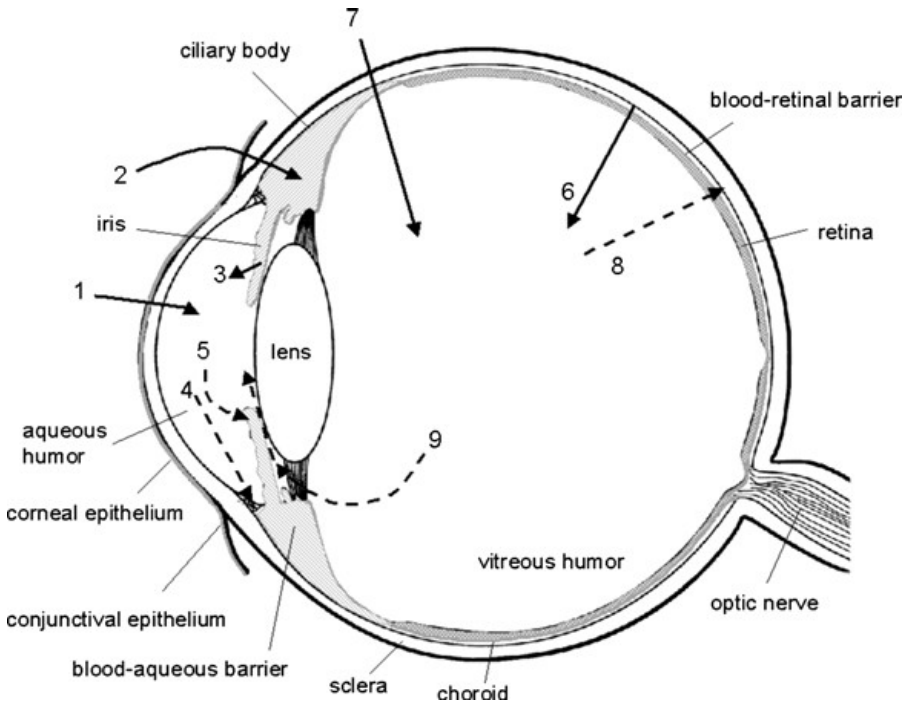


Fig 1 Schematic presentation of the ocular structure with the routes of drug kinetics illustrated. The numbers refer to following processes: 1) Tran corneal permeation from the lachrymal fluid into the anterior chamber, 2) Non-corneal drug permeation across the conjunctiva and sclera into the anterior urea, 3) Drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber, 4) Elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal, 5) Drug elimination from the aqueous humor into the systemic circulation across the blood-aqueous barrier, 6) Drug distribution from the blood into the posterior eye across the blood-retina barrier, 7) Intravitreal drug administration, 8) Drug elimination from the vitreous via posterior route across the blood-retina barrier, and 9) Drug elimination from the vitreous via anterior route to the posterior chamber.

1.2(b) Transcorneal penetration

Trans-corneal penetration mainly affected by corneal barrier, physicochemical properties of drug and active ion transport system present at cornea. Corneal epithelium is the main barrier for drug absorption in the eye. The stratified corneal epithelium act as a protective barrier against invasion of foreign molecule and also a barrier to ion transport. In a healthy corneal epithelium trans-cellular tight junction completely surround the most super facial cells. A tight junction serves as selective barrier for small molecules and they completely prevent the diffusion of macromolecules via Para cellular route, were as small molecules are able to penetrate through intercellular space of corneal epithelium. Corneal stroma is a highly hydrophilic tissue containing mostly water, and is a relatively open structure. Corneal stroma penetration rate is rate limiting step for lipophilic drug.

Hydrophilic drug penetrate primarily through Para cellular pathway which involves passive and active diffusion, while lipophilic drug prefers Trans-cellular route. For a topically applied drug passive diffusion by Trans-cellular/Para-cellular way is the main mechanism of permeation. Lipophilicity solubility, molecular size, charge and degree of ionization also affect the route and rote of penetration in cornea.

Various enzymes present in ocular tissue (protease, peptidase, and esterase) may metabolize many of ocular drugs during or after absorption. The corneal epithelium contains ionic channels that are selective for cation over anion and also contains an outwardly rectifying anion channel in the apical membrane and highly conductive potassium channel.

1.2(c) Non corneal absorption

This route involves drug penetration across the bulbar conjunctiva and underlying sclera in to the uveal tract and vitreous humor. This route is important for hydrophilic and large molecule with poor corneal permeability.

Tight junctions of spherical conjunctival epithelium are main barrier of drug penetration. conjunctival permeability of particular drug have magnitude higher than that of corneal penetration through sclera is mainly through perivascular spaces, through the aqueous media of gel like mucopolysaccharide or through spaces between collagen network. Sclera has more permeability compare to cornea.

1.3. Conventional ocular delivery constrains

For the ailments of the eye, topical administration is usually preferred over systemic administration so as to avoid systemic toxicity, for rapid onset of action, and for decreasing the required dose. Though topical administration offers many advantages to treat disorders of anterior structures of the eye, it suffers from a serious disadvantage of poor bioavailability due to several biological factors (Fig. 1), which exist to protect the eye and consequently limit the entry of ocular drugs. The constraints in topical delivery of the eye are discussed below.

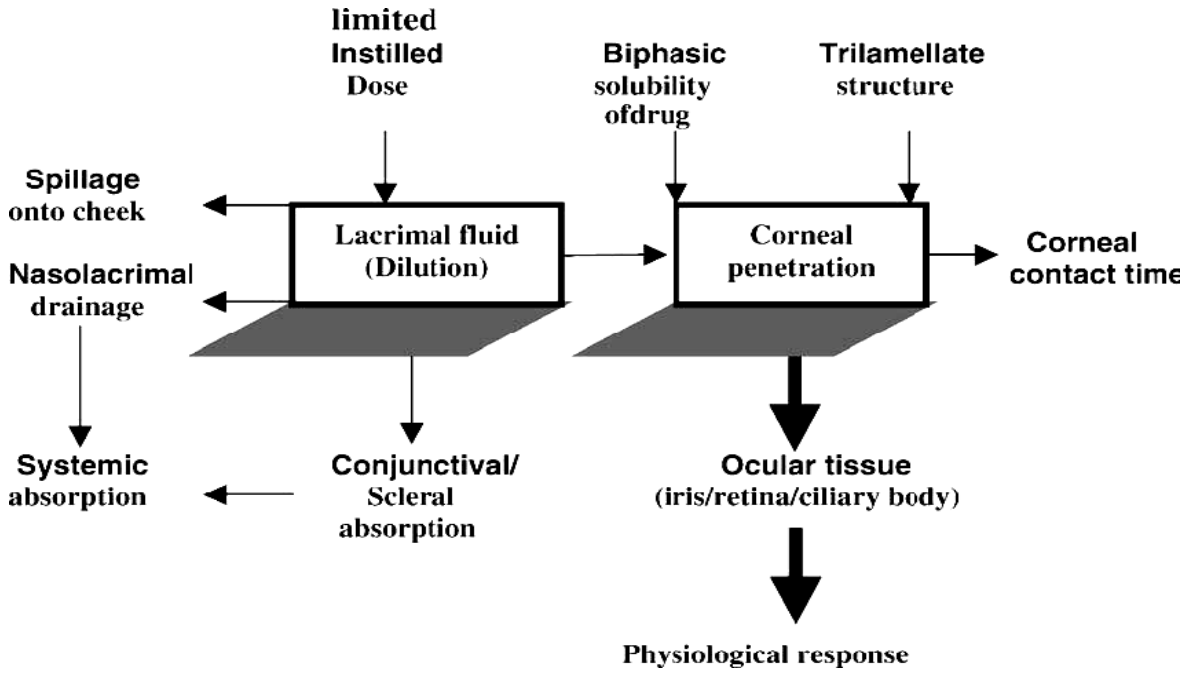


Fig .2. Factors attributing to poor bioavailability of an ophthalmic formulation.

1.4. **Disadvantage of topical ophthalmic formulations.**

The conventional topical ophthalmic preparations have the following disadvantages.

1) They have poor bioavailability because of

   a. Rapid precorneal elimination
   b. Conjunctival absorption
   c. Solution drainage by gravity
   d. Induced lacrimation
   e. Normal tear turn over

2) Frequent instillation of concentrated medication is required to achieve therapeutic effect.

3) Systemic absorption of drug and additives drained through nasolachrymal duct may result in undesirable effect.
4) The amount of drug delivered during external application may vary. The drop size of ocular medication is not uniform and dose delivered is generally not correct.

1.5. Requisites of controlled ocular delivery system

1. To over come the side effects of pulsed dosing (frequent dosing) and high concentration produced by conventional system.
2. To provide sustained and controlled drug delivery.
3. To increase the ocular bioavailability of drug by increasing cornel contact time. This can be achieved by effective coating or adherence to corneal surface, so that the released drug effectively reaches the anterior chamber.
4. To provide targeting within the ocular globe so as to prevent the loss to other ocular diseases.
5. To circumvent the protective barrier like drainage, lacrimation and diversion of exogenous chemicals into the systemic circulation by the conjunctiva.
6. To provide comfort and compliance to the patient and yet improve the therapeutic performance of the drug over conventional system.
7. To improve the better housing of the delivery system in the eye so as the loss to other tissues besides cornea is prevented.

Ocular drugs and delivery system are currently undergoing a process of design optimization due to inherent physiological and anatomical constraints of the eye leading to limited absorption topically applied drug.

1.6. **Formulation approaches to improve ocular bioavailability**

Various approaches that have been attempted to increase the bioavailability and the duration of therapeutic action of ocular drugs can be divided into two categories. The first is based on the use of the drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves, maximizing corneal drug absorption and minimizing precorneal drug loss. The conventional ocular aqueous solutions (eye drops), suspensions and ointments can be replaced by a more controlled, sustained and continuous drug delivery, using a controlled release ocular drug delivery system. These systems can achieve therapeutic action with a smaller dose and a fewer systemic and ocular side effects. Such systems include implantable systems, ocuserts, collagen shields etc., but the limitations of these systems include poor patient compliance, need of surgery, and difficulty in self-insertion.

Particulate drug delivery systems, like nanoparticles and microspheres, can also be used to improve the residence time of the drug. Upon administration to the eye, the particles reside at the delivery site and the drug is released from the particles through diffusion, chemical reaction, polymer degradation or ion-exchange mechanism. Smaller particles are better tolerated by the patients than larger particles and hence microspheres and nanoparticles represent very comfortable prolonged action ophthalmic drug delivery systems. However, some workers observed that nanoparticles consisting of poly (alkyl cyanoacrylate) damaged the corneal epithelium by disrupting the cell membrane.

Capacity of some polymers to adhere to the mucin coat covering the conjunctiva and the corneal surfaces of the eye by a non-covalent bond has been exploited to provide an intimate contact between the drug and the absorbing tissue, which may result in high

drug concentration in the local area and hence, drug flux through the absorbing tissue Common disadvantage observed is that the adhesive often detaches itself from the rate controlling drug delivery device and causes premature release of drugs.

Even though various drug delivery systems mentioned above offer a numerous advantages over conventional drug therapy but still they are not devoid of pitfalls, including

- Poor patient compliance and difficulty of insertion as in ocular inserts,
- Tissue irritation and damage caused by penetration enhancers and collagen shields,
- Toxicity caused by insertion of foreign substances, like albumin and polybutylcyanoacrylate, as in case of nanoparticles and microspheres
- Change in pharmacokinetic and pharmacodynamics of the drug as caused by altering the chemical structure of the drug (prodrug approach).

In order to overcome these problems, the researchers have come up with the concept of vesicular drug delivery systems as applied to corneal delivery. Vesicular delivery is a means of prolonged and controlled delivery. Drug enclosed in the lipid vesicles allows for an improved solubility and transport through the cornea.

1.7. Vesicular system in ocular delivery [21]

Vesicular systems not only help in providing prolonged and controlled action at the corneal surface but also help in providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action. The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. The rate of drug penetration depends not only on the physicochemical properties of the drug itself, such as its solubility and particle size, in case of suspensions but also on those of its vehicle. In vesicular dosage forms, the drug is encapsulated in lipid/surfactant vesicles, which can cross cell membrane. Vesicles therefore can be viewed as drug carriers and as such they change the rate and extent of absorption as well as the disposition of the drug. Vesicular drug delivery systems used in ophthalmic delivery broadly include liposomes and niosomes.

1.7.1. Liposomes

Liposomes are the microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 80 nm to 10 nm. Liposomes were first described by Bangham et al. Such vesicles composed of one or more phospholipid bilayer membranes can entrap both hydrophilic and hydrophobic drugs, depending on the nature of the drug and hence, it is possible to

apply water-insoluble drugs in liquid dosage form. According to their size, liposomes are known as either small unilamellar vesicles (SUV) (10–100 nm) or large unilamellar vesicles (LUV) (100–3000 nm). If more than one bilayers are present, then they are referred to as multilamellar vesicles. Liposomes offer advantages over most ophthalmic delivery systems in being completely biodegradable and relatively non-toxic. Another potential advantage of liposomes is their ability to come in an intimate contact with the corneal and conjunctival surfaces, thereby increasing the probability of ocular drug absorption. This ability is especially desirable for drugs that are poorly absorbed, for example, the drugs with low partition coefficient, poor solubility or those with medium to high molecular weights. Despite the above discussed factors, which make liposomes a potentially useful system for ocular delivery they are not very popular because of their short shelf life, limited drug capacity, and problems in sterilization. The latter problems can be taken as challenge to establish liposomes as an effective means of ocular delivery.

1.7.2. Niosomes.

Non-ionic surfactant based vesicles (niosomes) are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures (Fig. 4). The assembly into closed bilayers is rarely spontaneous and usually involves some input of energy such as physical agitation or heat. The result is an assembly in which the hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups enjoy maximum contact with same. These structures are analogous to phospholipid vesicles (liposomes) and are able to encapsulate aqueous solutes and serve as drug carriers. The low cost, greater stability and resultant ease of

storage of non-ionic surfactants has lead to the exploitation of these compounds as alternatives to phospholipids. Niosomes were first reported in the seventies as a feature of the cosmetic industry but have since been studied as drug targeting agents. These formulations use alternative materials to phospholipids such as Span 60, Span 40 and Span 20 which are inexpensive and widely available permitted food additives.

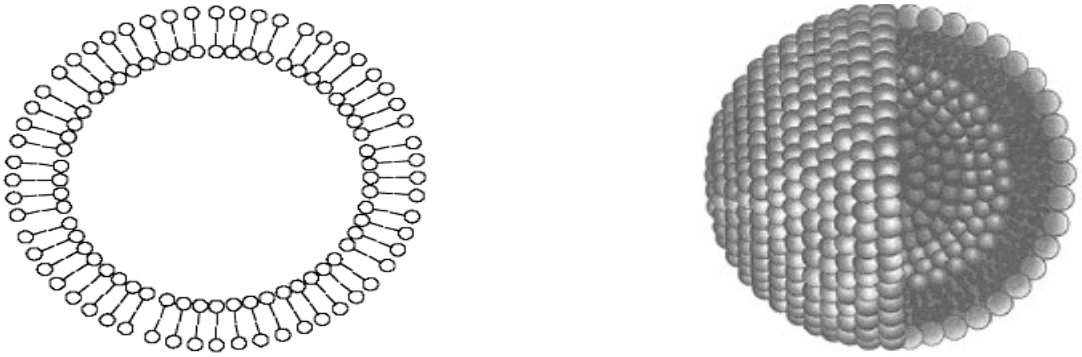


Fig. 3. Schematic representation of a niosome & 3-dimension structure

Niosomes are essentially nonionic surfactant based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulted from the organization of surfactant macro molecule as bilayer. Similar to Liposome, niosomes are formed on hydration of nonionic surfactant film, which eventually hydrates imbibing or encapsulating the hydrating aqueous solution. Compared to phospholipids used in the Liposome, the synthetic non ionic surfactants used in Niosome preparation are chemically stable, precise in chemical composition and cheaper in cost.

1.7.2(a) Physicochemical aspects of non ionic surfactant[21]

Structural components of niosomal bilayer

L'Oreal reported alkyl and dialkyl poly glycerol ethers as vesicles forming nonionic surfactant. Muller and Goymann 1987 investigated bilayer forming characteristics of PEG-polyglycols and polyethylene ethers system. Fig.4 shows a schematic representation of amphiphiles. In certain cases cholesterol is required in the formulation and vesicle aggregation or example may be prevented by the inclusion of molecules that stabilize the system against the formation of aggregates by repulsive steric or electrostatic effects. An amphiphiles forming niosome must possess a hydrophilic head group and a hydrophobic tail. The hydrophobic moiety may consist of one or two alkyl or perfluoroalkyl groups or in certain cases a single steroidal group. The alkyl group chain length is usually from C12–C18. Molecules may possess one, two or three alkyl chains. Perfluoroalkyl surfactants that form vesicles possess chain lengths as short as C10. Additionally crown ether amphiphiles bearing a steroidal C14 alkyl or C16 alkyl hydrophobic unit have been shown to form vesicles. While the number of hydrophobic permutations is at present limited, there have been a wide variety of hydrophilic head groups in vesicle forming surfactants and it is in this area of vesicle forming surfactant design that considerable scope for new formulations still exist. The two portions of the molecule may be linked via ether, amide or ester bonds.

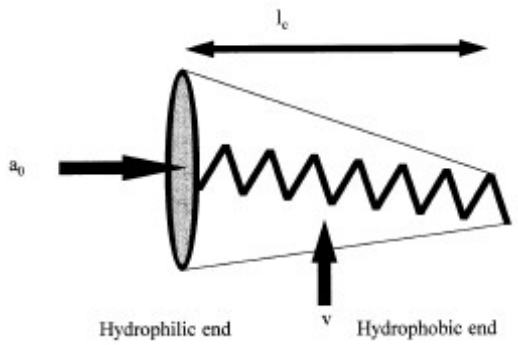


Fig. 4. Schematic representation of an amphiphile

We have observed that a parameter like the hydrophilic lipophilic balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. With the sorbitan monostearate (Span) surfactants, a HLB number between 4 and 8 was found to be compatible with vesicle formation. The guidance offered by the HLB number is useful as apart from the theoretical methods of estimating HLB number in which the relative proportions of both the hydrophilic and hydrophobic portions of the molecule are assessed, practical methods of HLB number determination have also been reported. These studies may be useful in the evaluation of new classes of compounds for their vesicle forming ability. The water soluble detergent polysorbate 20 also forms niosomes in the presence of cholesterol. This is despite the fact that the HLB number of this compound is 16.7 and it appears on first inspection to be too hydrophilic to form a bilayer membrane. However with an optimum level of cholesterol, it seems that niosomes are indeed formed from polysorbate. Although a particular membrane surfactant may be chosen by combining the hydrophilic moieties given in with an appropriate hydrophobic group. Established molecules may also be chosen from those mentioned in a few earlier reviews. Some of these surfactants such as the Span and Brij surfactants are already established pharmaceutical excipients. Span 60 (C18) and Span 40 (C16) gave the

greatest encapsulation efficiency for niosomes and were the least leaky niosomes due to the fact that these Span surfactants had the highest phase transition temperature. Also unsonicated Span 60 niosomes gave the greatest encapsulation efficiency. With sonicated Span surfactant niosomes the encapsulation efficiency followed the trend C18\C16\C12.

Table no. 1

Chemical specification of span 20, 40, 60, cholesterol, dicetylphosphate, and CF

Chemical Specification	Description	MW (g/mol)	MW Formula	HLB
Sorbitan monolaurate (Span20)	Clear viscous liquid	346.5	$C_{18}H_{34}O_6$	8.6
Sorbitan monopalmitate (Span40)	Yellowish power	402.6	$C_{22}H_{42}O_6$	6.7
Sorbitan monostearate (Span60)	White powder	430.6	$C_{24}H_{46}O_6$	4.7
Cholestrol	White powder	386.7	$C_{27}H_{46}O$	N/A
Dicetyl Phosphate	White powder	546.9	$C_{32}H_{67}O_4P$	N/A
5(6)-carboxyfluorescein	Yellowish powder	376.0	$C_{21}H_{12}O_7$	N/A

Membrane additives

Unfortunately the prediction of vesicle forming ability is not simply a matter of HLB numbers and chemical structure various other factors come into play. It is generally accepted that the parameters for self-assembly laid own by Israelachvili in which a critical packing parameter (CPP) was defined, largely hold true today.

$$CPP=v/lc\,a_o$$

Where v hydrophobic group volume, *l*c the critical hydrophobic group length and a_o the area of the hydrophilic head group (fig. 4.). A CPP of between 0.5 and 1 (the area of head group between 0.25-0.5) indicates that the surfactant is likely to form vesicles. A CPP of below 0.5 (indicating a large contribution from the hydrophilic head group area) is said to give spherical micelles and a CPP of above 1 (indicating a large contribution from the hydrophobic group volume) should produce inverted micelles, the latter presumably only in an oil phase, or precipitation would occur. Often various additives must be included in the formulation in order to prepare stable niosomes, which may be formed by the manipulation of the membrane forming agents in a typical system. The most common additive found in niosomal systems is cholesterol. Thus in cases where a mixture of surfactants or cholesterol is used to prepare niosomes, the operational CPP values will be those of the entire components.

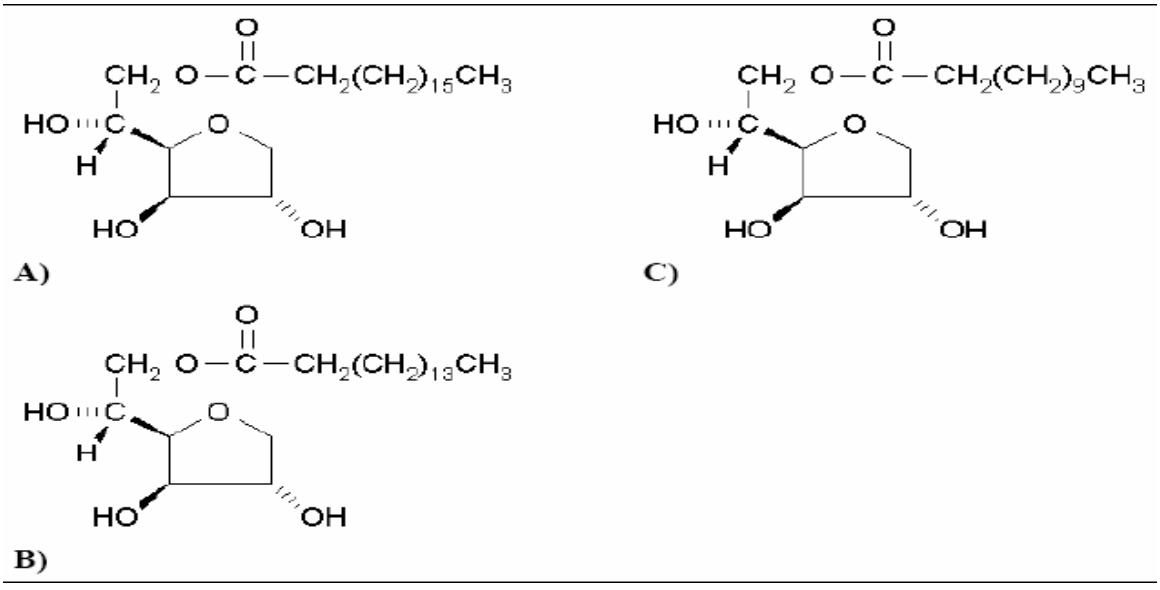


Fig.5. Chemical structure of span60 (A), span40 (B) and span20 (C)

Being amphipathic in nature cholesterol aligns itself in such a way the OH group orient towards aqueous phase while aliphatic chain align parallel to the hydrocarbon chain of surfactant. Further, in a mixed molecular bilayer it occupies an alternative position. The presence of rigid steroidal skeleton along side the carbon chain of surfactant could possibly restrict the freedom of movement of the carbon of the hydrocarbon segment thus providing an absolute rigidization. Also, the addition of cholesterol enables more hydrophobic surfactants to form vesicles, suppresses the tendency of the surfactants to form aggregates, and lends greater stability to the bilayer membranes by raising the gel liquid transition temperature of the vesicle[16]. Below are the phase transition temperatures of nonionic surfactants. As in the case of liposome the lipophilicity, temperature of hydration and phase transitions temperature are of importance in the vesicle formation.

Table no.2

Phase transition temperature of span 20, 40, and 60

Nonionic Surfactant	Acyl composition	Gel Transition Temperature
Sorbitan monolaurate (Span20)	C9	Liquid at room temperature
Sorbitan monopalmitate (Span40)	C13	46-47^{o}C
Sorbitan monostearate (Span60)	C15	56-58^{o}C

1.7.2(b) Methods of Preparation

Ether injection

The ether injection method essentially based on the slow injection of surfactant: cholesterol solution in ether through a 14 gauge needle at rate approximately 0.25 ml/min in to a preheated 4.0 ml aqueous phase maintained at 60^0 C. Fluorinated hydrocarbon evaporate at much lower temperature could be substituted ether in case where drug to be incorporated are highly susceptible to temperature. The variation in vesicular size in aqueous phase may be varied by an additional addition of the ethereal solution of surfactant whilst effective volume dispersion remains to be constant.

Hand shaking

Surfactant and cholesterol mixture in molar ratio dissolved in suitable quantity of organic solvent (diethyl ether, chloroform, alcohol) in around bottom flask. The ether is evaporated under vacuum at room temperature in a rotary flash evaporator. Upon hydration the surfactant swells and peeled of the support in to a film. The swollen amphiphiles eventually folds in to vesicles. The liquid volume entrapped into the vesicles appears small 5-10%. The entrapable volume seems to be unsuitable for water soluble drugs although the absolute yield per ml of solution per gram of surfactant may be satisfactory for practical purpose.

Sonication

Niosomes using sonications method prepared by baille et al where 150µ mol of surfactant cholesterol mixture was dispersed in 2 ml of aqueous phase in a vial. The dispersion was probe sonicated for 3 min at 60^0 C. essentially; the method involves the

formation of MLVs which are subsequently subjected to ultrasonic vibration. Probe sonaicator could be used if the sample size is small. However for large samples bath sonaicator is considered to be suitable. The finished vesicles are unilamellar in shape. Great care must be taken while working with temperature sensitive solute.

Reverse phase evaporation

Surfactants are dissolved in chloroform and 0.25 ml phosphate buffer saline is emulsified to get w/o emulsion. The mixture is then sonicated and subsequently chloroform is evaporated under reduced pressure. The surfactant forms gel first and subsequently hydrates to form vesicles.

Aqueous dispersion

The method deals with dispersion of vesicle forming agent and active drug which is subsequently homogenized at room temperature followed by continuous bubbling of nitrogen till the vesiculation or hydration completed. The bubbles possibly provide spherical gas at interface for amphiphiles to get organized as per thermodynamic stability requirements. Nitrogen is released subsequently and allows hydration of amphiphiles to form vesicles.

Extrusion

Niosomes prepared using C_{16} G_{12} a chemically defined non ionic surfactant by extrusion through a poly carbonate membrane (0.1 µm). It was found that using extrusion technique vesicle of mean size 138 nm could be prepared.

Separation of niosomes

The aqueous dispersion of solute bearing niosomes prepared by one of the above described method and is exhaustively dialyzed using cellophane tubing against phosphate buffer saline. The column chromatographic method also used for the separation of niosome from the free drug containing niosomal suspension. For vesicular systems, in order to make them free of the free drug sephadex G50 is commonly employed. The drug is preferentially retained in the column while vesicles percolate down the column.

1.7.2(c) Encapsulation efficiency and solute release rate

There is no successful and satisfactory study which shows a theoretical correlation of the entrapment efficiency of hydrophilic and hydrophobic solute within the niosomal vesicles. This is partly due to the method of preparation and subsequent processing of the system. However encapsulation efficiency can be said as a product of the stability of the dispersion. That is, the encapsulated solute and the solute retention capability of the encapsulating membrane, together with the stability of both the surfactant and vesicle structure, all contributes to the stability of the formulation. Vesicle loaded by trans-membrane gradient method shows higher entrapment efficiency. As a rule larger vesicles have higher entrapment compared to smaller one. Water soluble solutes results in increased vesicle size.

Encapsulation efficiency is a measure of solute retention and cholesterol has been shown to assist solute retention. The amount cholesterol also decides the release rate the extent of solute from the vesicle. Nevertheless there are some reports which show a decrease in the entrapment on inclusion of cholesterol. Similarly vesicles formed from

the polyoxyethylene 1, 2 disteroyl ether did not show any significant influence on encapsulation efficiency on inclusion of cholesterol.

The alky chain length also found to have clear influence on entrapment efficiency, as the chain length increases better the encapsulation of solute. An increase in the alky chain length of surfactant also found to show a gradual decrease in the vesicle size. The presence of surface charge on the vesicular dispersion is critical as it determines the aggregation character of the vesicles. Vesicular surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as zeta potential.

1.7.2(d) Niosome stability

Stable niosome dispersion must exhibit a constant particle size and a constant level of entrapped drug. There must be no precipitation of the membrane components, which are to a large extent not insoluble in aqueous media. It appears that the original size of the formulation has an effect on the stability of the system. This is in keeping with thermodynamic theory as the smaller niosomes require a higher input of energy and thus contain more excess energy and an inherently greater instability than the larger niosomes prepared by hand shaking. A further example of how the method of vesicle formation has important bearing on the stability of these systems is the fact that vesicles prepared by the solvent injection method (ethanol) are found to have an additional phase transition due to the presence of residual ethanol. A number of membrane properties rely on the temperature of the main phase transition such as the membrane permeability and rigidity. The introduction of membrane defects due to the presence of residual ethanol may destabilize these drug delivery systems. The addition of cholesterol found to increase the stability of the niosomal system by decreasing the leakage of system. The observed effect

of cholesterol is in accordance with the membrane stabilizing effect. Cholesterol found to decrease the fluidity of membrane which leads to membrane stabilization. It may be possible to stabilize niosomes by a variety of methods such as the addition of polymerized surfactants to the formulation, the use of membrane spanning lipids and the interfacial polymerization of surfactant monomers in situ.

1.7.2(e) Ocular Delivery [16]

Niosomes in topical ocular delivery are preferred over other vesicular systems because:

(i) They are chemically stable as compared to liposomes.

(ii) They can entrap both lipophilic and hydrophilic drugs.

(iii) They have low toxicity because of their non-ionic nature.

(iv) Unlike phospholipids, handling of surfactants requires no special precautions and conditions.

(v) They exhibit flexibility in their structural characterization, e.g. in their composition, fluidity, and size.

(vi) They can improve the performance of the drug via better availability and controlled delivery at a particular site.

(vii) They are biodegradable, biocompatible, and non-immunogenic.

An increased ocular bioavailability of water-soluble drugs entrapped in niosomes, (where liposomes are favorable as carrier system only for hydrophobic drugs) may be due to the fact that surfactants (chief constituents of niosomes) also act as penetration enhancers as they can remove the mucus layer and break junctional complexes. The irritation power of surfactants decreases in the following order: cationic > anionic > ampholytic > non-ionic so the non-ionic surfactants are preferred. The modified form of

niosomes known as discomes is also used in ophthalmic. The non-ionic surfactant vesicles have been reported to be successful, as an ocular vehicle for cyclopentolate independent of the pH, significantly improved the ocular bioavailability of cyclopentolate with respect to reference buffer solution and no irritation with the niosomal formulation was observed. Vyas et al[20] reported that there was an increase in the ocular bioavailability of timolol maleate encapsulated in niosomes as Compared to timolol maleate solution.

Drugs that could be considered for ocular delivery by vesicular systems, includes to anti glaucoma drugs, antibiotics and anti inflammatory (NSAIDS), which used in the treatment of anterior eye inflammation. The other drug includes the antiviral, antifungal and antiallergics.

REVIEW OF LITERATURE

1. Literatures on Vesicular Systems in Drug Delivery.

Jain *et al.,* [22] prepared Rifampicin niosome for lymphatic delivery using sorbitan esters and cholesterol 50:50. The drug entrapped vesicles characterized for their shape size and the drug entrapment efficiency and in vitro release, cumulative percent dose of drug in the lymphatic on intra peritoneal administration and intra venous administration were compared. The study revealed effective compartmentalization of drug after intra peritoneal administration of the niosome and the niosome could be successfully used for effective treatment if the tuberculosis along lymphatic.

Manosroi *et al.,* [15] studied on vesicles (niosomes) prepared with hydrated mixture of various non-ionic surfactants and cholesterol. The entrapment efficiencies of the vesicles and micro viscosities of the vesicular membrane depended on alkyl chain length of non-ionic surfactants and amount of cholesterol used to prepare vesicles. The stearyl chain (C18) non-ionic surfactant vesicles showed higher entrapment efficiency than the lauryl chain (C12) non-ionic surfactant vesicles. Niosome prepared with Tween 61 bearing a long alkyl chain and a large hydrophilic moiety in the combination with cholesterol at 1:1 molar ratio was found to have the highest entrapment efficiency of water soluble substances.

Lakshmi *et al.,* [23] studied on a novel formulation of niosome 0.25% methotrexate using a polymer Chitosan, which administered once daily for 12 weeks in comparison with placebo in patients with palmoplantar psoriasis. The results shows that the lesions treated with Niosomal Chitosan-Methotrexate formulation showed marked improvement in comparison to plain methotrexate and placebo gel in spite of twice a day application.

The results concludes that the 0.25% niosomal Methotrexate in Chitosan gel exhibited beneficial effect in psoriasis and did not exert any systemic toxicity.

Uchegbu *et al.,*[9] have prepared Doxorubicin niosomes (10 mg kg-1 doxorubicin) from sorbitan monostearate (Span 60), cholesterol and choleth-24 (24 oxyethylene cholesteryl ether) were administered intravenously to female NMRI mice bearing the MAC 15A subcutaneously implanted tumour. The results show that doxorubicin released from the niosomes was about 10 fold greater than the clearance of niosomal doxorubicin; the area under the plasma level-time curve increased 6 fold when doxorubicin was administered in niosomes, compared to doxorubicin solution and the modest tumour targeting was achieved by the delivery of doxorubicin in sorbitan monostearate niosomes, increasing the tumour to heart area under curve for 24 hours ratio from 0.27 to 0.36 and a doubling of tumoricidal activity. The overall level of doxorubicin metabolites was also increased.

Nasseri, [24] studied the mechanical characteristics of non-ionic bilayer membranes composed of sorbitan monostearate, cholesterol and poly-24-oxyethylene cholesteryl by measuring the modulus of surface elasticity (μ), a measure of membrane strength, as a function of cholesterol content and temperature. Study suggests an increase in the membrane elasticity on addition of cholesterol but excess of cholesterol above 40% mol leads to decrease in the surface elasticity. It was due to the formation of cholesterol clusters at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayers compromising their uniformity, strength and permeability.

Fang *et al.,* [6] studied on the skin permeation and partitioning in nude mouse skin of a fluorinated quinolone antibacterial agent, enoxacin in liposomes and niosomes. After topical application they observed the following permeation enhancer effect and the direct vesicle fusion with stratumcorneum, which may contribute to the permeation of enoxacin across skin. Formulation with niosomes demonstrated a higher stability after 48 h incubation compared to liposomes and the inclusion of cholesterol improved the stability of enoxacin liposomes according to the results from encapsulation and turbidity.

Tabbakhian *et al.,* [25] investigated enhancement of finasteride concentration at the pilosebaceous unit on topical application in the form of vesicles (liposomes and niosomes) as compared to finasteride hydroalcoholic solution. Liposomes consisted of phospholipid (dimyristoylphosphatidylcholine: cholesterol: dicetylphosphate) Niosomes comprised non-ionic surfactant (polyoxyethylene alkyl ethers (Brij® series) or sorbitan monopalmitate): cholesterol: dicetylphosphate. *In vitro* permeation of H-finasteride through hamster flank skin was faster from hydroalcoholic solution. *In vivo* deposition of H-finasteride vesicles in hamster ear showed that liquid-state vesicle were able to deposit the applied dose to the pilosebaceous unit studies, demonstrate the potentials of liquid-state liposomes and niosome for successful delivery of finasteride to the pilosebaceous unit.

Bandyopadhyay *et al.,* [26] studied on the self-organization of nonionic surfactant span 60 (sorbitan mono stearate) in presence of fatty alcohol (stearyl, cetyl and lauryl) Vesicular suspension had been characterized by transmission electron microscopy, dynamic light scattering, confocal laser scanning microscopy, dye entrapment and release

studies. Surface tension measurement indicates the suitability of fatty alcohols towards spontaneous vesicle formation from span 60.

Arunothayanun *et al.,* [27] describes an early prototype of a pulsatile delivery system for drug containing vesicles. Nonionic surfactant vesicles (niosomes) of average diameter 4-30 mm are extruded from glass capillaries using air pressures of 0.5-5 psi. Extrusion was affected by the size, shape, and membrane composition of the niosomes used. Spherical or polyhedral niosomes, formed by polyoxyethylene alkyl ethers with and without cholesterol, respectively, with diameters larger than the exit diameter of the capillary do not retain their membrane integrity on extrusion and were sheared to form new ultra structures. They observed pulsatile expulsion of groups of niosomes entrapping LHRH which indicates the feasibility of this system for pulsatile delivery of vesicles, although it requires miniaturization.

Girigoswami *et al.,* [28] found that niosomal vesicles are more stable than liposomal vesicles due to higher chemical stability of surfactants compared to phospholipids. Niosome had been prepared from Span20, Span80, Tween20 and Tween80. Fluorescence resonance energy transfer studies have been performed in these systems to determine donor–acceptor distances. It has been found that the fluorescence resonance energy transfer efficiency is better in niosome compared to micelles. The formation of niosome is guided by the hydrophile–lipophile balance value of the nonionic surfactant

Solanki *et al.,* [29] investigated the combined influence of 3 independent variables in the preparation of piroxicam proniosomes by the slurry method. A 3-factor, 3-level Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. The independent variables selected were molar ratio of Span 60: cholesterol, surfactant loading, and amount of drug. The transformed values of the independent variables and the Percentage drug entrapment (dependent variable) were subjected to multiple regressions to establish a full-model second-order polynomial equation. F was calculated to confirm the omission of insignificant terms from the full-model equation to derive a reduced-model polynomial equation to predict the percentage drug entrapment of proniosome-derived niosomes. Contour plots were constructed to show the effects of molar ratio of span 60: cholesterol, surfactant loading, and amount of drug on the percentage drug entrapment. A model was validated for accurate prediction of the percentage drug entrapment by performing checkpoint analysis. The Box-Behnken design demonstrated the role of the derived equation and contour plots in predicting the values of dependent variables for the preparation and optimization of piroxicam proniosomes.

Satturwar *et al.,* [30] prepared Ketoconazole niosomes prepared by ether injection technique using surfactant (Tween 40 or 80), cholesterol and drug in five different ratios by weight, The niosomes were characterized for size, shape, entrapment efficiency and in vitro drug release (by exhaustive dialysis). The formulations were also tested for *in vitro* (cup-plate method) and *in vivo* antifungal activity (in rabbits) and compared with free ketoconazole. The results of the present study indicate that niosomes have the potential to reduce the therapeutic dose of ketoconazole by improving its performance

Jagtap *et al.,* [31] formulated niosomes of Pentoxifylline characterized niosomes in terms of entrapment efficiency, particle size distribution, in vitro release and stability and investigate the bronchodilatory activity of plain and niosomal Pentoxifylline *in vivo* in guinea pigs. Pentoxifylline was entrapped in niosomes by lipid layer hydration method using span 60, cholesterol and dicetyl phosphate. The entrapment efficiency of niosomes of Pentoxifylline according to them is 9.26 plus minus 1.93 percent giving a sustained release of drug over a period of 24h and better stability over the period of storage. The plain and niosomal Pentoxifylline produced significant bronchodilatory effect in guinea pigs on histamine-induced bronchoconstriction. The study indicates that Pentoxifylline may be an effective bronchodilator.

Fang *et al.,* [5] studied the skin permeation of estradiol from various proniosome gel formulations across excised rat skin was investigated *in vitro*. The encapsulation efficiency and size of niosomal vesicles formed from proniosomes upon hydration were also characterized. Proniosomes with Span 40 and Span 60 increased the permeation of estradiol across skin. Niosome suspension (diluted proniosomal formulations) and proniosome gel showed different behavior in modulating transdermal delivery of estradiol across skin. Presence or absence of cholesterol in the lipid bilayers of vesicles did not reveal difference in encapsulation and permeation of the associated estradiol. The results show the importance of types and contents of non-ionic surfactant in proniosomes in the efficiency of transdermal estradiol delivery.

Pardakhty *et al.,* [32] prepared niosomes of polyoxyethylene alkyl ethers (Brij™) for encapsulation of insulin by film hydration method. Without cholesterol, brij 35 and brij 58 did not form niosomes, apparently because of relatively large polar head groups in comparison with their alkyl chains. The size of vesicles depended on the cholesterol content, charge incorporation or hydrophilicity of surfactants. Entrapment of insulin in

bilayer structure of niosomes protected it against proteolytic activity of chymotrypsin, trypsin and pepsin *in vitro.* The maximum protection activity was seen in brij 92/cholesterol vesicles. The kinetic of drug release for most formulations could be best described by Baker and Lonsdale equation indicating diffusion based delivery mechanism. These results indicate that niosomes could be developed as sustained release oral dosage forms for delivery of peptides and proteins such as insulin.

Arunothayanun *et al.,* [33] studied the physicochemical and biological differences between polyhedral and spherical: tubular niosomes polyhedral niosomes undergo a reversible shape transformation into spherical structures on heating above their phase transition temperature. The viscosity of polyhedral niosomes at room temperature is higher than their spherical counterparts due to their faceted and relatively rigid shape, At room temperature, polyhedral niosomes possess more rigid gel phase membranes and are less osmotically sensitive; however, they are more permeable because of a lack of or low levels of cholesterol in their membranes. Polyhedral niosomes loaded with luteinising hormone releasing hormone nonetheless, slow the release of drug compared to solution, albeit to a small extent.

Ismail A. Attia *et al.,* [34] prepared acyclovir niosomes in a trial to improve its poor and variable oral bioavailability. The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. The lipid mixture consisted of cholesterol, span 60, and dicetyl phosphate. The percentage entrapment was 11% of acyclovir used in the hydration process. The vesicles had an average size of 0.95 μm. Most of the niosomes have unilamellar spherical shape. *In vitro* drug release profile was found to follow

Higuchi's equation for free and niosomal drug. The niosomal formulation exhibited significantly retarded release compared with free drug. The *in vivo* study revealed that the niosomal dispersion significantly improved the oral bioavailability of acyclovir in rabbits after a single oral dose of 40 mg kg $^{-1}$. The niosomal dispersion showed significant increase in the mean residence time (MRT) of acyclovir reflecting sustained release characteristics. In conclusion, the niosomal formulation could be a promising delivery system for acyclovir with improved oral bioavailability and prolonged drug release profiles.

Dinesh Shenoy *et al.,* [35] investigated the *in vitro* release profile, stability and anti-fertility efficacy of some injectable, biodegradable formulations of Centchroman The formulations included an in situ gelling preparation namely Poly(Lactide-co-Glycolide) (PLGA)-in-Triacetin prepared by solution method and niosomes were prepared by lipid layer hydration method. These were evaluated for physicochemical characteristics like size distribution, percentage entrapment, *in vitro* drug release profiles and stability at different conditions of storage. *In vivo* anti-fertility activity was evaluated in female albino rats showing normal oestrous cycle by giving a single dose of the formulation histopathological sections of the uterus and ovary were also done. The formulations showed controlled drug release and enhanced stability whereas *in-vivo* studies showed promising anti-fertility activity for PLGA-in-Triacetin.

Kaur *et al.,* [16] reviews the constraints with conventional ocular therapy and explores various novel approaches, in general, to improve ocular bioavailability of the drugs, advantages of vesicular approach over these and the future challenges to render the vesicular system more effective. The discussion covers the importance of niosomes liposome, discomes etc in ocular delivery.

Carafa *et al.,* [36] formulated Non ionic surfactant vesicles entrapped lidocaine in the form of a free base and a hydrochloride. Non ionic surfactant vesicles were prepared from polyoxyethylene sorbitan monolaurate and cholesterol. The effect of vesicle composition and environmental pH condition on drug encapsulation efficiency was investigated. freeze-fracture microscopy technique, dynamic light scattering, permeation through Silastic and mouse abdominal skin, *in vitro* release kinetics of vesicle-entrapped drugs, fluorescence quenching analyses were also studied. Lidocaine HCl loaded vesicles showed a higher Permeation through mouse abdominal skin. Charged vesicles prepared in the presence of dicetylphosphate (DCP) and N-aetylpyridinium chloride showed low entrapment efficiency.

Almira *et al.,* [37] reported a novel method for rapid preparation of proniosomes with a wide range of surfactant loading. Slurry method has been developed by them to produce proniosomes using maltodextrin as the carrier. The time required to produce proniosomes by this simple method is independent of the ratio of surfactant solution to carrier material and appears to be scalable. The flexibility of the proniosome preparation method would allow for the optimization of drug encapsulation in the final formulation

based on the type and amount of maltodextrin. This formulation of proniosomes is a practical and simple method of producing niosomes at the point of use for drug delivery.

Sanyog *et al.,* [38] developed mannosylated niosomes as oral DNA vaccine carriers for the induction of humoral, cellular and mucosal immunity. Niosomes composed of span 60, cholesterol and stearylamine as constitutive lipids were prepared by reverse phase evaporation method and were coated with a modified polysaccharide o-palmitoyl mannan. The coated niosomes were found to be better stabilized in simulated GIT conditions. The immune stimulating activity was studied by measuring serum anti-HBs Ag titer, secretory IgA level in intestinal and salivary secretions. O-palmitoyl mannan coated niosomes produced humoral (both systemic and mucosal) and cellular immune response upon oral administration. The study signifies the potential of OPM coated niosomes as DNA vaccine carrier and adjuvant for effective oral immunization.

Beugin *et al.,* [39] have synthesized and characterized. Monomethoxypoly (ethylene glycol) cholesteryl carbonates (M-PEG-Chol) with polymer chain molecular weights of 1000 (M-PEG1000-Chol) and 2000 (M-PEG2000-Chol). Their aggregation behavior in mixture with diglycerol hexadecyl ether (C16G2) and cholesterol has been examined by cryotransmission electron microscopy, high-performance gel exclusion chromatography, and quasielastic light scattering. Nonaggregated, stable unilamellar vesicles were obtained at low polymer levels with optimal shape and size homogeneity at cholesteryl conjugate/ lipids ratios of 10 mol% M-PEG1000-Chol. Higher levels up to 30 mol% lead to the complete solubilization of the vesicles into disc-like structures of decreasing size with increasing PEG content. This study underlines the bivalent role of M-PEG-Chol

derivatives, while behaving as solubilizing surfactants; they provide an efficient steric barrier, preventing the vesicles from aggregation and fusion.

Naresh *et al.,* [40] attempts to activate and exploit macrophages in delivering niosomal and thermosensitive niosomal bleomycin more quantitatively to tumor site using niosome encapsulated immunomodulators muramyl dipeptide and tuftsin. Niosomal bleomycin and thermosensitive niosomal bleomycin were prepared by lipid layer hydration method. The antitumor efficacy was assessed using two tumor models viz. Sarcoma-180 and Ehrlich ascites using Balb/C mice. Accumulation of higher bleomycin levels after macrophage activation exerted increased antitumor effect. They suggest more quantitative delivery of bleomycin encapsulated in niosomes, to the tumor site is possible after macrophage activation.

Namdeo *et al.,* [41] prepared niosomes of 5-fluorouracil by the hand shaking method, reverse phase evaporation and ether injection method using a series of Spans. Span 40 and span 60 showed a much sustained drug release. Introduction of cholesterol and dicetyl phosphate decreased the vesicle size and extended the release. The niosomal formulation shows increased half life AUC and a decrease in the volume of distribution. They suggest vesicles of 5 Fluorouracil as a promising drug deliver system.

Shahiwala *et al.,* [42] developed niosomal based trans-dermal delivery system of nimesulide, niosome prepared by lipid hydration technique using tweenss and spans. The formulations characterized for higher entrapment efficiency were incorporated in to 1% Carbapol gel. The highest percentage oedema inhibition observed with niosomal gel. Their investigation conclusively demonstrates prolongation of drug release and increase

in the amount of drug retained in to the skin and permeation across the skin after niosomal encapsulation of nimesulide. Niosomal gel also demonstrated an enhanced anti inflammatory activity compared to plain drug gel and marketed formulation.

Parthasarathi *et al.,* [43] prepared niosome encapsulated vincristine sulfate by transmembrane pH gradient. Drug uptake process (remote loading method) was evaluated for toxicity and antitumour activity after administration to tumour bearing mice. The toxicity of vincristine sulfate was reduced after niosome encapsulation and anticancer activity improved, which may be due to better delivery of vincristine at the tumour site.

Uchegbu *et al.,* [44] prepared niosome from a hexadecyl diglycerol ether a hexadecyl poly 5 oxyethylene ether sorbitan monosterate in 1:1 ratio with cholesterol .span 60. Niosomes were found to be stable both in 4^{o}C and 24^{0}C retaining 75% of encapsulated material after 28 days. The incubation of niosome in plasma revealed the higher stability of it in plasma and the results suggest the possible release of drug only in the target site, that is drug available in vivo only after the cleavage of vesicle.

Oommen *et al.,* [45] entrapped methotrexate complexed with b-Cyclodextrin into niosomes by lipid layer hydration method. To characterize niosomes for different physicochemical properties and to investigate the potential of niosome entrapped methotrexate b-cyclodextrin complex in tumour treatment. The entrapment efficiency of the complex within the niosomes was determined by separating the un-entrapped drug using dialysis. The niosomal entrapment efficiency was higher in the case of methotrexate b-cyclodextrin complex than with the plain drug. Comparison of the drug release in phosphate buffer revealed a relatively slow release pattern of the entrapped

drug complex from the vesicles as compared to plain MTX encapsulated niosomes. Better stability on storage was also observed with the niosome entrapped complex. The complex entrapped niosomes produced an improved anticancer activity as evident by enhanced volume doubling time and growth delay.

Rentel *et al.,* [46] developed a peroral vaccine delivery system based on non-ionic surfactant vesicles (niosomes) were evaluated using BALB:c mice. Ovalbumin was encapsulated in various lyophilized niosome preparations consisting of sucrose esters, cholesterol and dicetyl phosphate. Two different formulations were compared in this study. The specific antibody titres within serum, saliva and intestinal washings were monitored by ELISA on days 7, 14, 21 and 28 after intragastric administration niosomes resulted in a significant increase in antibody titres. Administration of ovalbumin and empty niosomes did not exert a similar effect; neither did administration of any control formulation. In contrast to ovalbumin loaded Wasag 7 niosomes, application of the more hydrophilic Wasag 15 niosome preparations did not result in an increase in antibody titres.

Amelie Bochot *et al.,* [47] designed an ocular delivery system based on the dispersion of liposomes into a thermo sensitive gel made of a copolymer of ethylene oxide and propylene oxide (poloxamer 407). In order to stabilize liposomes in the gel, PEG2000-DSPE was introduced. Adsorption studies investigated by size and ζ-potential, measurements have shown that the adsorption was higher for positively charged or neutral non- sterically stabilized liposomes. Results also shown that liposome permeability was dramatically reduced in the presence of poloxamer 407, when PEG-

DSPE was incorporated into the liposomes. The study suggests new system based on a dispersion of pegylated liposomes into thermo sensitive poloxamer 407 is proposed, offering new potentialities for delivery of drugs.

Elzainy *et al.,* [48] Studied the peripheral H_1-antihistaminic activity and extent of systemic absorption of cetirizine from liposomes applied to the skin. Cetirizine was incorporated into small unilamellar vesicles (SUV) and multilamellar vesicles (MLV) prepared using L-α-phosphatidylcholine hydrogenated (HPC), and into Glaxal Base (GB) as the control. In a randomized, crossover study, each formulation, containing 10 mg of cetirizine, was applied to the depilated backs of 6 rabbits (3.08 ± 0.05 kg). Histamine-induced wheal tests and blood sampling were performed before cetirizine application and at designated times for up to 24 hours afterwards. Compared with baseline, histamine-induced wheal formation was suppressed by cetirizine in SUV only at 24 hours, in MLV from 0.5 to 24 hours, and in GB from 0.5 to 8 hours.

2. Vesicular Systems in Ocular Delivery

Gholam A. Peyman *et al.,* [49] had a discussion on delivery systems for intra ocular route. In brief; lntravitreal drug delivery has been developed to treat posterior segment diseases because the blood-ocular barrier prevents treatment by topical, systemic, or sub-conjunctival routes from attaining therapeutic levels in the vitreous. Endophthalmitis, uveitis, proliferative vitreoretinopathy, and viral retinitis are treated by intravitreal injection. Efforts to sustain drug delivery have included encapsulation of drugs in liposomes (made of lipids) or microspheres (made of polymers). In many instances the drug's toxicity to the retina was reduced and the clearance time was slowed. However,

these methods cause clouding of the vitreous and can prolong drug delivery for only one month. Implantable devices have been used, such as an osmotic minipump, a drug pellet coated with polyvinyl alcohol and ethylene vinyl acetate, and polysulfone capillary fiber. Biodegradable devices are under investigation, including a drug matrix and a porous reservoir system, both made of polymers; these devices would not require surgical removal.

Rania M. Hathout *et al.,* [50] prepared reversephase evaporation (REVs) and multilamellar (MLVs) acetazolamide liposomes consisting of egg phosphatidylcholine (PC) and cholesterol (CH) in molar ratio with or without stearylamine (SA) or dicetyl phosphate (DP) as positive and negative charge inducers, respectively. The prepared liposomes were evaluated for their entrapment efficiency and in vitro release. Multilamellar liposomes entrapped greater amounts of drug than REVs liposomes. Drug loading was increased by increasing CH content as well as by inclusion of SA. Drug release rate showed an order of negatively charged > neutral > positively charged liposomes, which is the reverse of the data of drug loading efficiency. From physical stability data the positively charged liposomes are less leaky neutral liposome retained least drug after the storage period of 3 months at 4^0C. The intraocular pressure (IOP)-lowering activity of selected acetazolamide liposomal formulations was determined and compared with that of plain liposomes and acetazolamide solution. Multilamellar acetazolamide liposomes revealed more prolonged effect than REVs liposomes. The positively charged and neutral liposomes exhibited greater lowering in IOP and a more prolonged effect than the negatively charged ones.

Vyas *et al.,* [20] have prepared non-ionic surface active agents based discoidal vesicles (discomes) bearing timolol maleate incorporated with Solulan C24 in order to effect vesicle to discome transition. The prepared system characterized for size, shape and drug release profile in vitro. They were found to release the contents following biphasic profile particularly in the case where the drug was loaded using a pH gradient technique. The prepared system could produce or sustain a suitable activity profile upon administration into the ocular cavity; however, systemic absorption was minimized to a negligible level. The discomes were found to be promising and of potential for controlled ocular administration of water-soluble drugs.

Aggarwal *et al.,* [51] prepared Chitosan or Carbapol coated niosomal timolol maleate formulation by reverse phase evaporation technique using span 60 and compared with the timolol maleate solution 0.25% in terms of in vitro release and I.O.P lowering. *In-vitro* release was extended significantly by the in corporation of the niosome and the further by polymer coating. The 0.25% timolol maleate niosomal formulation compared with the 0.5% marketed gel formulation the result showed the niosomal preparation significantly better.

Law *et al.,* [52] investigated the *in vitro* corneal penetration and *in vivo* corneal absorption of acyclovir from an acyclovir-containing liposome system. Results of in vitro corneal penetration demonstrated that positively charged liposomes resulted in a penetration rate lower than those of negatively charged liposomes and free acyclovir in solution. An *in vivo* study indicated that the extent of acyclovir absorption from positively charged liposomes was higher than those from negatively charged liposomes

and free acyclovir. The acyclovir concentration in the cornea after administration of positively charged liposomes was greater than those of negatively charged liposomes and free acyclovir. From morphological observation of the cornea surface treated with liposomes, it was suggested that positively charged liposomes formed a completely coated layer on the cornea surface leading to an increase of residence time. Therefore, positively charged liposomes resulted in an increase of acyclovir absorption.

Chetoni *et al.,* [53] have studied ocular pharmacokinetics of topically administered acyclovir liposome in comparison with commercial acyclovir ointment. The acyclovir liposomal dispersion produced a significantly higher drug concentration profile in the aqueous humor with respect to the three reference formulations containing the same acyclovir concentration. *In vitro* release tests substantiated the concept that positively charged liposomal formulations owe their efficacy to interact with the positively charged corneal epithelium.

Budai *et al.,* [54] developed ciprofloxacin containing liposomes and gel formulation to minimize the tear driven dilution in the conjunctival sac a long pursued objective of the ophthalmology. Physicochemical properties (pH, osmolarity, viscosity, expandability, membrane fluidity) and *in vitro* release of were studied. Electron paramagnetic resonance spectroscopy studied to find the molecular interaction; the polymeric hydrogel (Poly methacrylic acid 940NF) in the preparation ensured the steady and prolonged ciprofloxacin release. In addition, encapsulation of ciprofloxacin in to the liposomes prolonged the *in-vitro* release of the anti bacterial agent depending on the lipid composition (α-L DPPC and lecithin).

Guinedi *et al.,* [55] prepared niosomes from Span 40 or Span 60 and cholesterol in the molar ratios of 7:4, 7:6 and 7:7 using reverse-phase evaporation and thin film hydration methods. The prepared systems were characterized for entrapment efficiency, size, shape and *in vitro* drug release. Stability studies were carried out to investigate the leaching of drug from niosome during storage. The intraocular pressure (IOP) lowering activity of acetazolamide niosomal formulations in rabbit was also studied. Higher entrapment efficiency reported by span 60 vesicles, the niosomes retained 75% of drug on storage in the refrigerated condition for 3 months. Span 60 and cholesterol in a 7:4 molar ratio were found to be the most effective and showed prolonged decrease in IOP and showed only a slight ocular irritation.

Diebold *et al.,* [56] evaluated *in vitro* and *in vivo* a colloidal nano-system with the potential to deliver drugs to the ocular surface. They prepared nano-system, liposome chitosan nanoparticle complexes, as a complex between liposomes and chitosan nanoparticles. The conjunctival epithelial cell line was exposed to several concentrations of three different liposome chitosan nanoparticle complexes to determine the cytotoxicity. Conjunctival epithelial cells were examined by confocal microscopy. Eyeball and lid tissues from liposome chitosan nanoparticle complexes treated rabbits were evaluated for the *in vivo* uptake and acute tolerance of the nanosystems. Strong cellular uptake liposome chitosan nanoparticle *in vivo* and less intensive uptake by the corneal epithelium was reported. These data demonstrate those liposome chitosan nanoparticles are potentially useful as drug carriers for the ocular surface.

Omaima *et al.,* [57] formulated a liposomal preparation of acetazolamide to be applied topically, and evaluated it for *in vitro* and *in vivo* performance. Acetazolamide liposomes were prepared using the reverse phase evaporation technique. Neutral, positively-charged and negatively-charged liposomes were evaluated for their entrapment efficiency, drug release, and *in vivo* activity. The percent entrapment was higher with positively charged liposome and least with negatively charged one. The proportion of drug released after 9 hr was 13.36%, 33.8% and 26.7% for negatively-charged, neutral and positively-charged liposomes respectively. They found a good correlation between the percent of inhibition of carbonic anhydrase activity and the amount of drug released.

Law *et al.,* [58] investigated loading efficiency and release characteristics of acyclovir-containing liposomes. It was found that positively charged liposomes gave the highest loading efficiency. The neutral liposomes showed a loading efficiency in between those of the positively charged and negatively charged liposomes. Liposomes prepared by the method of drug–lipid film hydration presented a higher loading efficiency than that prepared by lipid film hydration with drug solution. For positively charged liposomes, the release rate was faster at higher molar ratios of stearylamine. However, for negatively charged liposomes, the release rate with a molar ratio of dicetylphosphate at 0.15 was greater than at 0.3. Decrease of mobility was found for positively charged liposomes after loading with acyclovir, whereas, no significant change in mobility was observed for negatively charged and neutral liposomes.

Thirumurthy *et al.,* [59] evaluated the retinal toxicity of plain and liposomal formulation of flucanazole at various dose levels after intravitreal injection. The study conducted in the New Zealand albino rabbit revealed that plain flucanazole at a concentration of 100 µg and above caused retinal changes, with disorganization of the photoreceptor outer segments. However, liposome formulation of fluconazole did not show any significant microscopic changes of the retina.

Aggarwal *et al.,* [60] prepared niosomes of acetazolamide (by reverse phase evaporation method) and coated with Carbopol for the latter's bioadhesive effect. The pharmacodynamic studies showed 33% fall in IOP with the developed formulation, and the effect was sustained for 6 hr after instillation. The aqueous humor disposition of the drug from the developed bioadhesive coated niosomal formulation (ACZREVbio) is compared with the aqueous suspension of the drug (containing 1% (w/v) Tween 80 as a dispersing agent) at similar concentrations. The concentration of acetazolamide absorbed in the aqueous humor at various times from the control suspension and from ACZREVbio was determined by microdialysis in male albino rabbits. The peak concentration of drug absorbed in the aqueous humor from the ACZREVbio formulation was almost two times of that obtained with the equivalent amount of acetazolamide control suspension. The aqueous humor disposition indicates peaks and troughs in drug concentration which may be related to the decrease in aqueous humor formation, such that the drug concentration or volume increased at these points.

Barber *et al.,* [61] investigated on tear fluid induced release of liposome entrapped agents. Rabbit tear fluid was shown to promote the release of both an entrapped water soluble dye (5-carboxyfluorescein) and a high molecular weight protein (acetylcholinesterase) from multilamellar liposome. Heat treatment of tear fluid was found to have no effect on the release of the enzyme but did reduce dye leakage. Pre-incubation of the tear fluid with empty liposome was found to have no significant effect on either enzyme or dye release. Unlike serum where liposome destabilization is reportedly almost entirely the result of high density lipoproteins, our results suggest that multiple factors are involved in tear-mediated liposome destabilization.

3. Literatures on Diclofenac Sodium.

Raja Naresh *et al.,* [62] entrapped diclofenac sodium in niosomes comprising of Tween 85 and Tween 85 poloxamer F 108 mixture. Anti- inflammatory efficacy of these niosomes was compared with that of free diclofenac sodium in adjuvant induced arthritic rats. It was found that the niosomal diclofenac sodium formulations prepared by employing a 1:l combination of Tween 85 and poloxamer F 108 elicits a better and consistent anti-inflammatory activity for more than 72 hours after administration of a single dose.

Turker *et al.,* [63] describes a novel approach for designing drug delivery systems for intra-articular treatment of rheumatoid arthritis by liposome, niosome, lipogelosome and niogelosome formulations of diclofenac sodium. Retention of these systems was evaluated by radio labeling with Tc-99m and gamma scintigraphy in arthritic rabbits. The study results show that radio-labeled lipogelosome formulation containing diclofenac

sodium retained much longer in the experimentally arthritic knee joints of the rabbits. Great retention of diclofenac sodium in the arthritic joint said to reduce potential adverse systemic effects of the drug because of local administration into the diseased area. The study concludes as it is a promising drug delivery system for Intra-articular drug delivery.

Kao Xiang Sun *et al.,* [64] prepared diclofenac sodium cationic liposomes by reverse-phase evaporation method and the formula of liposome were optimized with uniform design. HPLC method was established and validated for the determination of diclofenac sodium in precornea, cornea and aqueous humor of rabbit eye. Liposome and eye drop solution 50 microL with total 50 microg diclofenac sodium were instilled to eyes of rabbits, separately. Samples of tear, cornea and aqueous humor were collected at different time intervals after rabbits were sacrificed. The ocular pharmacokinetics was investigated by the concentration-time data of tear, cornea and aqueous humor. Diclofenac sodium cationic liposomes can increase the corneal contact time, enhance the corneal permeability of diclofenac sodium and improve its ocular bioavailability.

Katrin Kriwet *et al.,* [65] studied the relation ship between the colloidal structure of a topical formulation and the drug release *in vitro* as well the influence of the microstructure on the stratum corneum permeability. The study revealed drug transport across from the aqueous solution and from vesicles with a high effective diffusion co efficient is controlled by stratum corneum. The intact liposome entrapped flouromicrography of cryo-section of human skin showed no deep penetration of diclofenac diethyl amine from intact liposome.

Hwang *et al.,* [66] examined Remote loading of the model drugs diclofenac, insulin and fluorescein isothiocyanate labeled insulin (FITC-insulin) into liposomes by formation of trans-membrane gradients. A trapping efficiency of almost 100% was obtained for liposomal diclofenac, by the calcium acetate gradient method, where as liposomes prepared by the conventional reverse-phase evaporation vesicle method had low trapping efficiencies. Soybean-derived sterol was reported to be better stabilizer of the dipalmitoylphosphatidylcholine bilayer membrane than cholesterol. The work has demonstrated a remote loading method for weak acids such as diclofenac into liposomes by the acetate gradient method. From the result of remote loading of FITC-insulin into liposomes by the pH gradient method, this method reported for the preparation of liposomal peptides.

Szucs *et al.,* [67] conducted a prospective, randomized, double blinded, placebo-controlled clinical trial. Consenting consecutive patients 49 in numbers with corneal were randomly assigned to receive either diclofenac or control vehicle drops. Pain relief was measured using a visual Numeric Pain Intensity Scale (NPIS) before and after treatment. 25 of the patients received diclofenac and 24 received control vehicle drops. Both groups were similar in gender, age, pretreatment pain duration, NPIS score and analgesic use. There was significantly greater improvement in the 2-hour NPIS score in the diclofenac group compared with the control group. Study concludes by saying diclofenac ophthalmic solution appears to be a safe and effective analgesic in the treatment of traumatic corneal abrasions.

Gennaro D'Angelo *et al.,* [68] evaluated the efficacy of prolonged treatment with preservative-free diclofenac sodium 0.1% eye drops in patients with vernal keratoconjunctivitis. Study was performed in 22 patients with vernal keratoconjunctivitis treated with preservative-free diclofenac sodium 0.1% eye drops. Patients used the eye drops four times daily in both eyes for 120 days. Forty per cent of the patients showed an improvement in their symptoms at the end of the treatment. Total signs and symptoms scores were significantly decreased at the end of treatment compared with the baseline values. Significant decreases in conjunctival redness, itching and photophobia were observed at the end of treatment. Conjunctival hyperaemia was significantly reduced at the end of treatment, while no significant differences were observed for corneal lesions and for papillary size. No patient showed exacerbation of the disease during the treatment. Study demonstrates the efficacy and safety of preservative-free diclofenac sodium 0.1% eye.

Reddy *et al.,* [69] compared the effect of diclofenac sodium 0.1% following cataract surgery was to routine corticosteroid, dexamethasone phosphate 1% in a prospective, double-blind randomized study. Groups were similar in baseline parameters. Postoperative inflammatory response, intraocular pressure and best-corrected visual acuity following standard extra capsular cataract extraction were assessed in both groups in the initial 21 days and the severity of these parameters was graded. The two groups did not differ statistically in treatment effect for any of the variables including aqueous cells, flare, ciliary congestion, Descemet's folds, visual acuity and intraocular pressure. However, there seemed to be a trend towards quicker improvement with corticosteroid

when cells in the anterior chamber were considered. There were no side effects from topical diclofenac, and it was well tolerated.

Kocak *et al.,* [70] performed a clinical double-blind study in patients who had undergone extra capsular cataract extraction and intraocular lens implantation to compare the anti-inflammatory effects of diclofenac sodium 0.1% and flurbiprofen 0.03% eye drops. The diclofenac group included 21 eyes of 21 patients and the flurbiprofen group included 22 eyes of 22 patients. The parameters compared were pachymetry of the cornea, corneal surface changes, intraocular pressure and the degree of inflammation of the anterior chamber at one, three and six weeks after cataract surgery. There was no statistically significant difference between the two treatment groups in corneal pachymetry, corneal surface changes and the anterior chamber inflammation. Both drugs were well tolerated and may be safely used to reduce inflammation for cataract surgery.

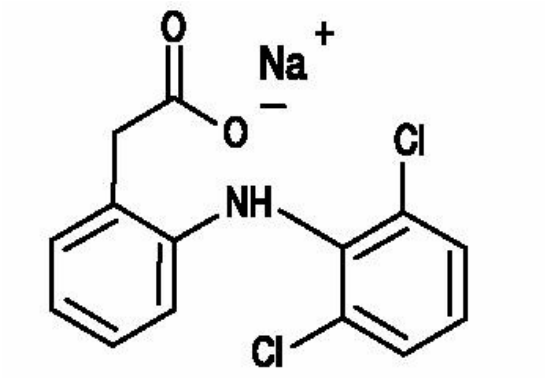
Carl P. Herbort *et al.,* [71] compared the anti-inflammatory effect of topical diclofenac sodium 0.1% in a fixed combination with gentamicin 0.3% to the anti-inflammatory effect of dexamethasone phosphate 0.1% in a prospective randomized double masked double-dummy study in patients undergoing cataract surgery. Eighty seven patients were recruited, 45 being assigned to the diclofenac group and 42 to the dexamethasone control group. Diclofenac was significantly better than dexamethasone at controlling flare at day 3 and day 12–14. Mean anterior chamber cells were also significantly lower at day 12–14 and day 28. The commonest adverse event was transient punctate keratitis, which occurred in 15 diclofenac and 3 dexamethasone patients. While both treatments were effective at controlling post-operative inflammation, the diclofenac-

gentamicin combination followed by diclofenac alone was significantly better at suppressing flare and cells but showed a slightly higher incidence of punctate keratitis and eye discomfort.

Palmero *et al.,* [72] studied the ocular pharmacokinetics of topical diclofenac sodium in two experimental models of ocular inflammation, and compared to physiological conditions. Keratitis or uveitis was induced by intrastromal injection of clove oil or by intravitreal lipopolysaccharide in rabbits. The control eyes were not inflamed, simultaneously to the induced of inflammation 30 µl of 0.1% diclofenac were applied topically in the right eye. In physiological conditions, diclofenac reached a peak level in the cornea and iris ciliary body (ICB) at 30 min slowly decreasing afterwards. Low levels of diclofenac were found in AH. In keratitic eyes Diclofenac concentrations in keratitic AH and ICB were lower than in controls. In uveitic eyes, corneal and ICB levels peaked at 30 min, being significantly higher than in controls and decreased quickly to very low levels at 1 h after application. In uveitic AH, diclofenac levels were lower than in controls. Plasma levels were very low in all experimental groups. It is concluded that the ocular pharmacokinetics of topical diclofenac is affected by inflammatory processes in the eye, reaching higher levels in the target tissues.

DRUG PROFILE[73-80]

Diclofenac Sodium



Formula:

$C_{14}H_{11}Cl_2NO_2Na$

IUPAC name:

2- [2-(2, 6-dichlorophenyl) aminophenyl] ethanoic acid

Molecular weight:

318.14 g

Category:

Anti inflammatory, Analgesic

Description:

Faintly yellow white to light beige, slightly hygroscopic crystalline powder

Melting point:

280^0C

Solubility:

Freely soluble in Methanol, sparingly soluble in Water, very slightly soluble in Acetonitrile and insoluble in Chloroform and 0.1 N Hydrochloric acid.

Indication:

Diclofenac sodium 0.1% ophthalmic solution indicated for treatment of post operative inflammation in patient who have under gone cataract extraction, and temporary relief of pain and photophobia in patients under gone corneal refractive surgery.

Dose:

Cataract surgery: One drop should be applied to the affected eye, four times daily, beginning 24 hours after surgery and continuing through out the first two weeks of the post operative period.

Corneal refractive surgery: One or two drops should be applied to the operative eye within the hour prior to surgery, within 15 minutes after surgery, one or two drops should be added to the operative eye and continued 4 times daily for up to 3 days.

Mode of action:

It has analgesic, antipyretic and anti inflammatory activities. It is an inhibitor of cyclooxygenase and its potency is substantially greater than that of indomethacin and naproxen or several other agents. In addition diclofenac sodium appears to reduce intracellular concentration of free arachidonic acid in leukocytes perhaps by altering the release or up take of fatty acid.

Pharmacokinetics:

Diclofenac sodium rapidly and completely absorbed on oral administration; peak concentration in plasma reaches within 2-3 hours. Administration with food slows the rate but doesn't alter the extent of absorption. There is substantially first pass metabolism such that only about 50% of diclofenac sodium is available systematically. The drug is extensively bound to plasma protein(99%) and its half life in plasma is 1-2 hours. The volume of distribution of drug is 0.17 liters/kg. Diclofenac sodium accumulates in synovial fluid after oral administration, which may explain the duration of therapeutic action of that is considerably higher than the plasma half life. Diclofenac sodium is metabolized in the liver by cytochrome P-450 isozyme of CYP2C subfamily. 2-hydroxydiclofenac, the principle metabolite and other hydroxylated forms; after glucoronidation and sulfation the metabolite were excreted in urine (65%) and bile (35). 0.01% is the total fraction of drug that excreted through urine in its original form. Plasma levels of diclofenac sodium following ocular instillation of two drops of diclofenac sodium ophthalmic solution, 0.1% to each eye were below the limit of quantization (10 ng / mL) over a 4-hour period. This study suggests that limited, if any, systemic absorption occurs with diclofenac sodium ophthalmic solution, 0.1%.

Contra indication:

- Hypersensitivity against Diclofenac sodium.
- History of allergic reactions (bronchospasm, shock, rhinitis, urticaria) following the use of Aspirin or another NSAID
- Third-trimester pregnancy

- Active stomach and/or duodenal ulceration or gastrointestinal bleeding
- Inflammative intestinal disorders such as Crohn's disease or ulcerative colitis
- Severe insufficiency of the heart (NYHA III/IV) Recently, a warning has been issued by FDA not to treat patients recovering from heart surgery
- Severe liver insufficiency (Child-Pugh Class C)
- Severe renal insufficiency (creatinine clearance <30 ml/min)
- Caution in patients with preexisting hepatic porphyria, as diclofenac sodium may trigger attacks
- Caution in patients with severe, active bleeding such as cerebral hemorrhage

Adverse reaction

Diclofenac sodium is among the better tolerated NSAIDs. Though 20% of patients on long-term treatment experience side effects, only 2% have to discontinue the drug, mostly due to gastrointestinal complaints.

Ocular: Transient burning and stinging was reported in 15% of patients with the use of topical diclofenac sodium ophthalmic solution, 0.1% In cataract studies, keratitis occurred in 28% of patients receiving diclofenac sodium ophthalmic solution 0.1%, however, most of the cases of keratitis occurred prior to drug therapy. Elevated intraocular pressure was reported in 15% of patients receiving diclofenac sodium ophthalmic solution, 0.1%. The following adverse reactions were reported in approximately 5% or less of the patients: abnormal vision, anterior chamber reaction,

blurred vision, conjunctivitis, corneal deposits, corneal edema, corneal lesions, corneal opacity, discharge, injection, iritis, irritation, itching and ocular allergy**.**

Systemic: The following adverse reactions were reported in 3% or less of the patients: abdominal pain, asthenia, chills, dizziness, facial edema, fever, headache, insomnia, nausea, pain, rhinitis, viral infection, and vomiting

Precautions:

General: It is recommended that Diclofenac Sodium Ophthalmic Solution, 0.1%, like other NSAIDs, be used with caution in surgical patients with known bleeding tendencies or who are receiving other medications that may prolong bleeding time. Diclofenac Sodium Ophthalmic Solution, 0.1% may slow or delay healing. Diclofenac Sodium Ophthalmic Solution, 0.1% has no significant effect upon intraocular pressure; however, elevations in intraocular pressure may occur following cataract surgery.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long term therapy in rats by oral diclofenac sodium up to 2 mg/kg/day (approximately the human oral dose) has revealed no significant increases in tumor incidence. There was a slight increase in benign rat mammary fibro adenomas in mid-dose females (high dose females had excessive mortality) but the increase was not significant for this common rat tumor. In mice employing oral diclofenac sodium up to 2 mg/kg/day for 2 years did not reveal any oncogene potential. Diclofenac sodium did not show mutagenic potential in various mutagencity studies including the Ames test. Diclofenac sodium administered to male and female rats at 4 mg/kg/day did not affect fertility.

Teratogenic effect: In mice at oral doses up to 5000 times (20 mg/kg/day) and in rats and rabbits at oral doses up to 2500 times (10 mg/kg/day) of the human topical dose have revealed no evidence of teratogenicity due to diclofenac sodium, despite the induction of maternal toxicity and fetal toxicity. In rats, maternally toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival. Diclofenac sodium has been shown to cross the placental barrier in mice and rats.

Nonteratogenic effects: Because of the known effects of prostaglandin biosynthesis-inhibiting drugs on the fetal cardiovascular system (closure of the ductus arteriosus), the use of Diclofenac Sodium Ophthalmic Solution, 0.1% during late pregnancy should be avoided.

Pediatric: Safety and effectiveness in pediatric patients have not been established.

Over dose

The maximum daily dose of diclofenac sodium is 200 mg. Over dosage will not ordinarily cause acute problems. If accidentally ingested, fluids should be taken to dilute the medication.

RESEARCH ENVISAGED

1. Aim of Work

Diclofenac Sodium, a drug which widely accepted for its safety and efficacy in the treatment of anterior segment eye inflammation and as prophylaxis, against cystoid's macular edema is used in this study. The low bioavailability, low solubility and short half life of this drug make it a candidate for control delivery.

Niosomes, synthetic microscopic vesicles consisting of an aqueous concentration enclosed in a bilayer consisting of cholesterol and one or more nonionic surfactants, have been reported as a possible approach to improve the low corneal penetration and bioavailability characteristic shown by conventional ophthalmic vehicles.

In this work a potential drug delivery system of diclofenac sodium entrapped niosomes for ocular delivery have been developed and characterized.

2. Plan of Work

The present work was carried out to prepare and evaluate niosomal drug delivery system of diclofenac sodium with non ionic surfactant, span 60, in various proportions. The following experimental protocol was therefore designed to allow a systemic approach to the study.

- Procurement of drug and raw materials.
- Preparation of standard curves.
- Formulation of niosomes.
- Evaluation of niosomes for the following physico-chemical parameters.
    - Microscopy.
    - Encapsulation efficiency and drug loading.
    - *In-vitro* release study.
    - Drug release kinetic data analysis.
    - Stability study.
    - Test for significance.
    - Zeta potential analysis.
    - *In- vivo* drug release study.

MATERIALS AND INSTRUMENTS

Materials Used

The following materials were used for the research work in their best quality available.

Table no. 3

Materials used for the research work

S.No.	Name	Grade	Company Name
1.	Diclofenac sodium	Pharma	Novaratis Limited, India
2.	Cholesterol	L.R	Loba Chem Pvt. Ltd., Mumbai
3.	Span 60	L.R	Loba Chem. Pvt. Ltd., Mumbai
4.	Chloroform	L.R	Loba Chem Pvt. Ltd., Mumbai
5.	Methanol	L.R	S.D.Fine Chem Ltd., Mumbai
6.	Sodium acetate	L.R	Loba Chem Pvt. Ltd., Mumbai
7.	Ethyl acetate	L.R	S.D.Fine Chem Ltd., Mumbai
8.	Sodium chloride	L.R	Loba Chem Pvt. Ltd., Mumbai
9.	Potassium di-hydrogen phosphate	L.R	S.D.Fine Chem Ltd., Mumbai
10.	Sodium hydroxide	L.R	Nice chemicals Pvt. Ltd., Cochin
11.	Di. Sodium hydrogen phosphate	L.R	S.D. Fine Chem Ltd., Mumbai
12.	Xylocaine 4%	L.R	Astra Zenica Ltd., India
13.	Distilled water	L.R	Leo Scientific, Erode
14.	H.P.L.C. water.	H.P.L.C	Qualigens, India

Table no. 4

Instruments used for the research work

S.No.	Name	Company Name
1.	Rotary flash evaporator	Super fit, India ltd.
2.	Cooling centrifuge	Remi Motors Ltd, Mumbai.
3.	Electronic digital balance	Schimadzu, Japan.
4.	HPLC system	Schimadzu, Japan.
5.	Dialysis membrane 50	Hi media, India
6.	pH meter.	Systronioc, Chennai
7.	Double beam UV/VIS Spectrophotometer.	ELICO, Mumbai
8.	Zeta Potential Probe (model DT-300)	Zetasizer 4, France.

EXPERIMENTAL PROCEDURES

1. Niosome Preparation [42]

The niosome formulations were prepared by lipid film hydration technique. Drug (Diclofenac sodium), non ionic surfactant and cholesterol were weighed (surfactant: cholesterol in µmol) and dissolved in chloroform methanol (2:1) in a 100 ml round bottom flask. A thin lipid film was formed under reduced pressure in a rotary flash evaporator. The film was then hydrated by 10 ml of PBS[1] pH 7.4 at room temperature with gentle shaking. The niosome suspension further hydrated up to 24 hrs at 2-8^0 C. The stabilized MLVs were used for further studies.

Table no. 5

Compositions of Niosomal batches of Diclofenac sodium

Formulation No.	Ratio(µmol) (surfactant: cholesterol)	Surfactant (mg)	Cholesterol (mg)
F_1	200:200	86	77.32
F_2	200:175	86	67.66
F_3	200:150	86	57.9
F_4	200:125	86	48.32
F_5	200:115	86	44.45
F_6	200:100	86	38.66
F_7	200: 85	86	32.8
F_8	200: 75	86	28.19
F_9	150:200	64.5	77.32
F_{10}	150:150	64.5	57.9
F_{11}	150:125	64.5	48.32

Drug content used 10 mg per batch

1 2.38 gm of Di-sodium hydrogen phosphate, 0.19gm of potassium di-hydrogen phosphate and 8.0 gm of sodium chloride were weighed accordingly and made up to 1000 ml with distilled water.[79]

2. Characterization of Niosomes

Preparation of standard curve of Diclofenac sodium in PBS

100 mg of Diclofenac sodium was dissolved in 100 ml of pH 7.4 phosphate buffer saline solution to the concentration of 1000µg/ml. 1 ml of this solution was taken and made up to 100 ml with buffer solution which contains the concentration of 10 µ g/ml, 1 to 10 ml were taken from this solution and made up to 10 ml to get the concentration ranges of 1 to 10µg/ml. The absorbance of the above said solutions were measured against the phosphate buffer saline pH 7.4 as blank at 275 nm using UV spectrophotometer.[63] Then the calibration curve was plotted taking concentration on X-axis and absorbance on Y-axis.

2.1. Entrapment efficiency [81]

Niosome entrapped diclofenac sodium was estimated by dialysis method. The prepared niosomes were placed in the dialysis bag 50 (presoaked for 24 hrs). Free diclofenac sodium was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 7.4. The dialysis of free diclofenac sodium always completed after 12-15 changes, when no Diclofenac was detectable in the recipient solution. The dialyzed diclofenac sodium was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 275 nm. The samples from the bulk of solution diluted ten times before going for absorbance measurement. The free diclofenac sodium in the bulk of solution gives us the total amount of un-entrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

$$\text{Percent Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

2.2. Microscopy

The vesicle formation by the particular procedure was confirmed by optical microscopy in 400x resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera.

2.3. *In-vitro* drug release study

Preparation of standard curve of diclofenac sodium

Preparation of pH 7.4 Phosphate Buffer [80]

50.0 ml of 0.2 M potassium di-hydrogen phosphate was placed in a 200 ml volumetric flask, added the specified volume of 39.1 ml of 0.2 M sodium hydroxide and then made up to the volume by water.

Potassium di-hydrogen phosphate, 0.2 M

27.218 gm of potassium di-hydrogen phosphate was dissolved in distilled water and diluted to 1000 ml.

0.2M sodium hydroxide solution

8 gm of sodium hydroxide was dissolved in distilled water and diluted to 1000ml.

Standard Curve of Diclofenac Sodium

100mg of diclofenac sodium was dissolved in 100ml of pH 7.4 phosphate buffer solution which gives the concentration of 1000µg/ml. 1ml of this solution was taken and made up to 100ml with buffer solution which contains the concentration of 10 µg/ml, 1 to 10ml were taken from this solution and made up to 10ml to get the concentration ranges of 1 to 10µg/ml. The absorbance of the above said solutions were measured against the phosphate buffer pH 7.4 as blank at 280 nm using UV spectrophotometer. [65] Then the calibration curve was plotted taking concentration on X-axis and absorbance on Y-axis.

Release Study [51]

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. 2 mg equivalent of 0.1% of niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 100 ml simulated tear fluid (pH7.4 phosphate buffer). The beaker was placed over magnetic stirrer and the temperature was maintained at 37 ± 1^0C. 5 ml samples were withdrawn periodically and were replaced by fresh buffer. The sink condition was maintained through out the experiment. The withdrawn samples were diluted two times and analyzed for drug content using U.V. spectrophotometer at 280 nm keeping phosphate buffer pH 7.4 as blank.

2.3.1 Drug Release Kinetic Data Analysis.

The release data obtained from various formulations were studied further for their fitness in the zero order release pattern. The study was conducted by checking the fitness of data in models like Higuchi's and peppa's.

2.4. Stability Study

2.4.1. Physical stability [55]

Physical stability study was carried out to investigate the leaching of drug from niosome (in a suspension) during storage. Best three (F_4, F_5, F_6) of the optimized diclofenac niosomal suspension composed of span-60 and cholesterol sealed in glass vials and stored in refrigerated temperature(2-8^{0}C) for a period of 3 months. Samples from each batch were withdrawn after the definite time intervals and the residual amount of drug in the vesicles was determined. Stability data of three formulations were further analyzed for significant difference by paired t-test.

2.4.2 Zeta potential analysis

Zeta potential was analyzed to measure the stability of niosome by studying its colloidal property. The study was conducted using zeta potential probe (model DT-300). The formulation F_6 which was found to have a better physical stability, was further analyzed by this method for its vesicular stability.

2.5 *In Vivo* Study [83-86]

Male albino rabbits 10-12 weeks old, weighing 2.5-3.5 kg were used in the present study. They were housed individually with husk bedding and fed with standard pellet diet and water as much required. The temperature was maintained at 28$\pm$2^{0}C through out the study. The study protocol was approved by Institutional Animal Ethical Committee for the use of animal in research (Proposal No.NCP/IAEC/PG/03/2007-2008).

Two healthy rabbits were used for the study. Three drops of 0.1% niosomal suspension of diclofenac sodium was instilled in the lower cul-de-sac of each eye. The upper eyelids were gently held closed for 10 seconds to maximize the corneal contact. At the 4^{th} and 8^{th} hour of post dose, eyes were anesthetized using 4% Xylocaine solutions topically and the aqueous humor was sampled from 4 eyes by using a 26 gauge needle. The aqueous humor from each eye extracted by introducing a 26 gauge needle between the junction of sclera and cornea. After the extraction the eyes were treated with ciprofloxacin eye drops for the prevention of infection. Sampled Aqueous humor was then mixed with 100 µl of ethyl acetate and kept in the refrigerator for one hour. The mixture was then centrifuged at 3000 rpm for 20 minutes and the supernatant obtained was analyzed for the presence of diclofenac sodium by HPLC- U.V detector, by comparing with the retention time of a standard solution (50 µg/ml).

Apparatus for chromatographic condition

Qualitative estimation of diclofenac sodium was done by HPLC. Filtered and degassed mixture of methanol and sodium acetate 0.1 M (60:40) was used as mobile phase. The equipments include following, Shimdazu HPLC pump and detector, Phenominax $C_{18,}$ 5µ column (250x4.6mm) and fixed volume injector (10µl). The mobile phase delivered in rate of 1 ml/min the effluents analyzed in 254 nm. A single 50µg/ml solution of drug in the mobile phase injected as sample. A blank was run after each injection to ensure the reliability.

RESULT AND DISCUSSION

Various niosomal formulations of diclofenac sodium using span 60 and cholesterol were prepared. The prepared formulations were further characterized for the percentage drug entrapment by dialysis method.

1. Entrapment Efficiency

1.1 Standard curve of diclofenac sodium in phosphate buffer saline pH 7.4

Table no. 6

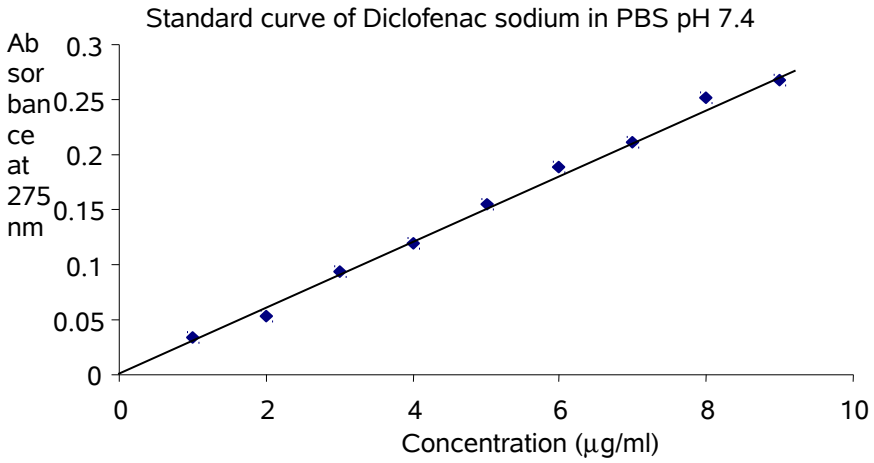
Standard curve of diclofenac sodium in PBS pH 7.4

S.No.	Concentration µg/ml	Absorbance at 275 nm
1	1	0.034
2	2	0.054
3	3	0.094
4	4	0.120
5	5	0.155
6	6	0.189
7	7	0.211
8	8	0.251
9	9	0.268

R^2 = 0.988

Slope = 0.0285

Graph no. 1



1.2 Entrapment efficiency of various formulations

Entrapment efficiency was studied for all the 11 formulations to find the best in terms of entrapment efficiency. Higher entrapment efficiency of the vesicles of span 60 is predictable because of its higher alkyl chain length. The entrapment efficiency was found to be higher with the formulation no. F_6 (82.10%), which may have an optimum cholesterol surfactant ratio to provide a high entrapment of diclofenac sodium. The niosomal formulations having high surfactant concentration (F_6, F_4 and F_5) have the higher entrapment efficiency which might be due to the high fluidity of the vesicles. Very low cholesterol content (F_8) was also found to cause low entrapment efficiency (57.89%), which might be because of leakage of the vesicles. It was also observed that very high cholesterol content (F_9) had a lowering effect on drug entrapment to the vesicles (44.91%). This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. The higher entrapment may be explained by high cholesterol content (~50% of the total lipid). There are reports that entrapment efficiency was increased, with increasing cholesterol content and by the usage of span-60 which has higher phase transition temperature. The larger

vesicle size may also contribute to the higher entrapment efficiency. Entrapment efficiency showed by various formulations are specified in Table no.7.

Table no. 7

Entrapment efficiency of various formulations

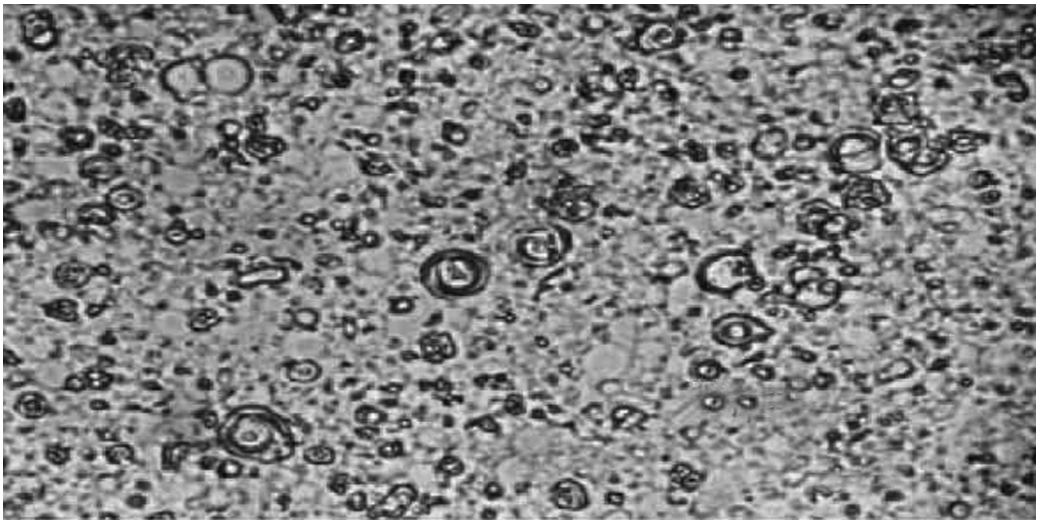
S.No.	Formulation no	Molar ratio (span60 : Cholesterol)	Entrapment efficiency* %
1	F_1	200:200	62.10 ± 1.32
2	F_2	200:175	63.85 ± 1.27
3	F_3	200:150	68.07 ± 0.47
4	F_4	200:125	70.87 ± 0.32
5	F_5	200:115	77.89 ± 0.88
6	F_6	200:100	82.10 ± 0.92
7	F_7	200: 85	63.86 ± 0.41
8	F_8	200: 75	57.89 ± 0.58
9	F_9	150:200	44.91 ± 1.18
10	F_{10}	150:150	67.10 ± 0.75
11	F_{11}	150:125	58.45 ± 0.52

*Average of three formulations, ± S.D.

2. MICROSCOPY

The prepared vesicles were studied under 400x magnification to observe the formation of vesicles. Some unevenness of vesicles that observed under the study may be due to drying process under normal environment condition. The photomicrograph of niosomes is shown in the figure below. The particles found to be uniform in size and shape.

Figure no. 6 Photomicrograph of niosome in a dry glass slide



3. *IN-VITRO* RELEASE PROFILE

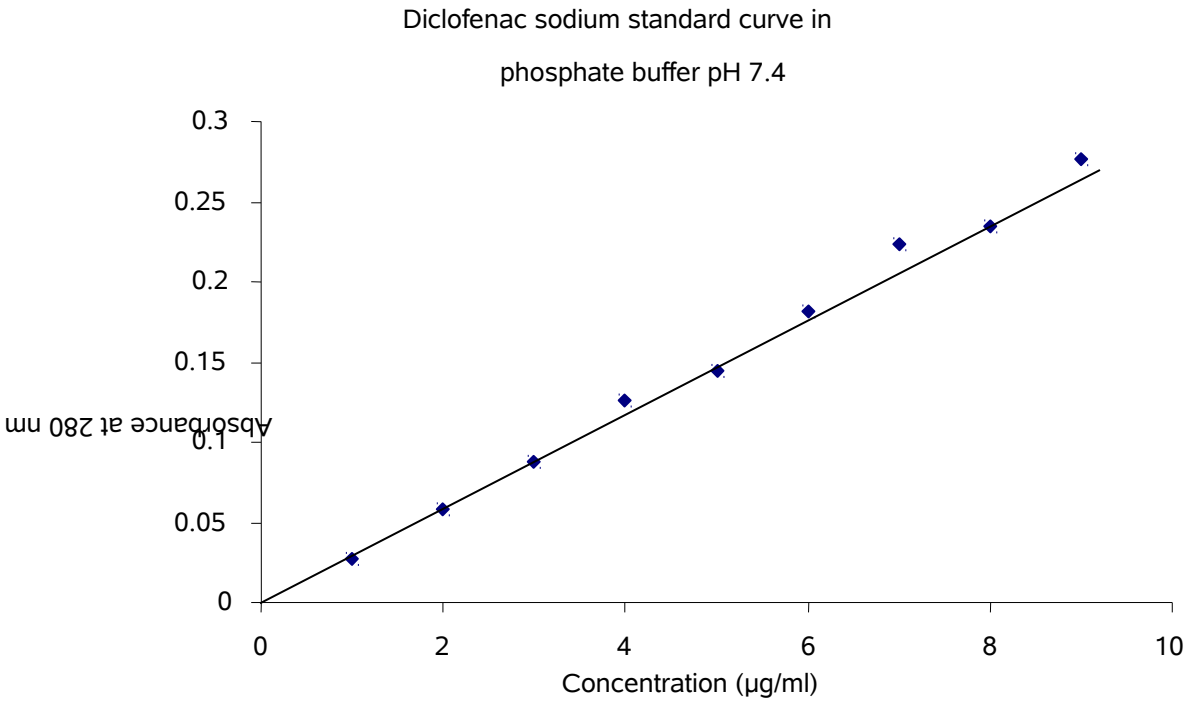
3.1. Standard curve of Diclofenac sodium in phosphate buffer pH 7.4

Table No.8

Standard cure of Diclofenac sodium in phosphate buffer pH 7.4

S.NO	Concentration	Absorbance at 280 nm
1	1 µg/ml	0.027
2	2 µg/ml	0.058
3	3 µg/ml	0.088
4	4 µg/ml	0.126
5	5 µg/ml	0.145
6	6 µg/ml	0.182
7	7 µg/ml	0.223
8	8 µg/ml	0.235
9	9 µg/ml	0.276

Graph no.3



Slope: 0.0308 R^2 : 0.997

3.2. Release profile of various formulations.

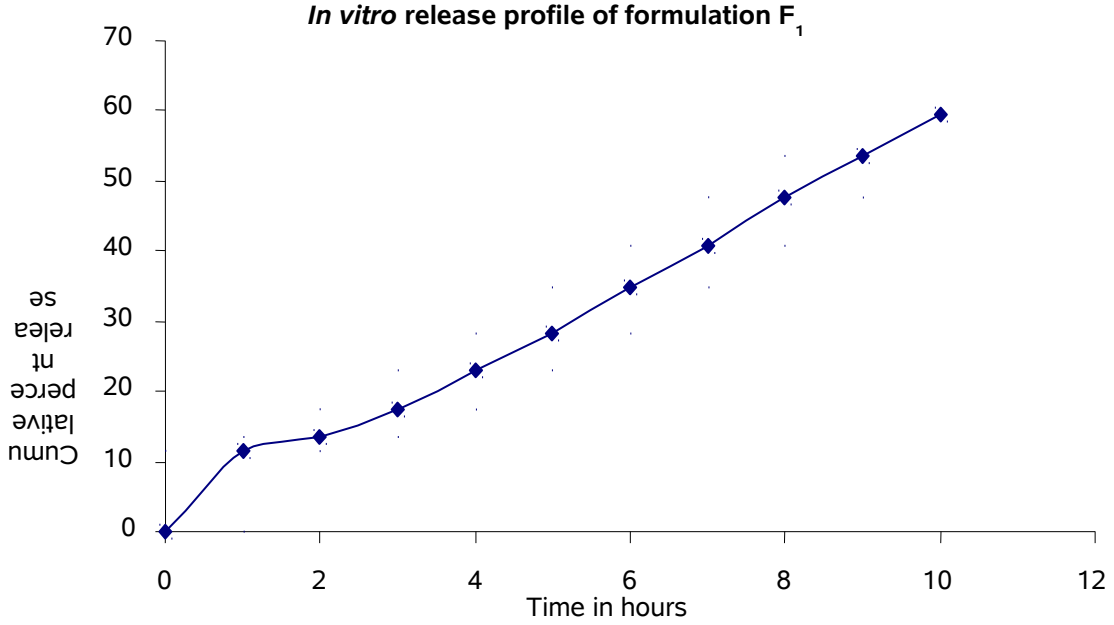
The release study was conducted for all the 11 formulations. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 60% release within a period of 10 hours. The formulations which have high cholesterol ratio ($F_{9,}$ F_{10}) were found to sustain the drug release. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment. The above specified three best formulations F_4, F_5, and $F_{6,}$ were found to give a cumulative release of 64.04%, 64.17% and 70.01% respectively over a period of 10 hrs, the higher release from the formulation F_6 may be because of its low cholesterol content. Formulations F_1, F_9 and F_{10} having the highest cholesterol content showed the lowest release over 10 hours, they provide a release of 59.42%, 52.47% and 56.43% respectively.

Table No.9

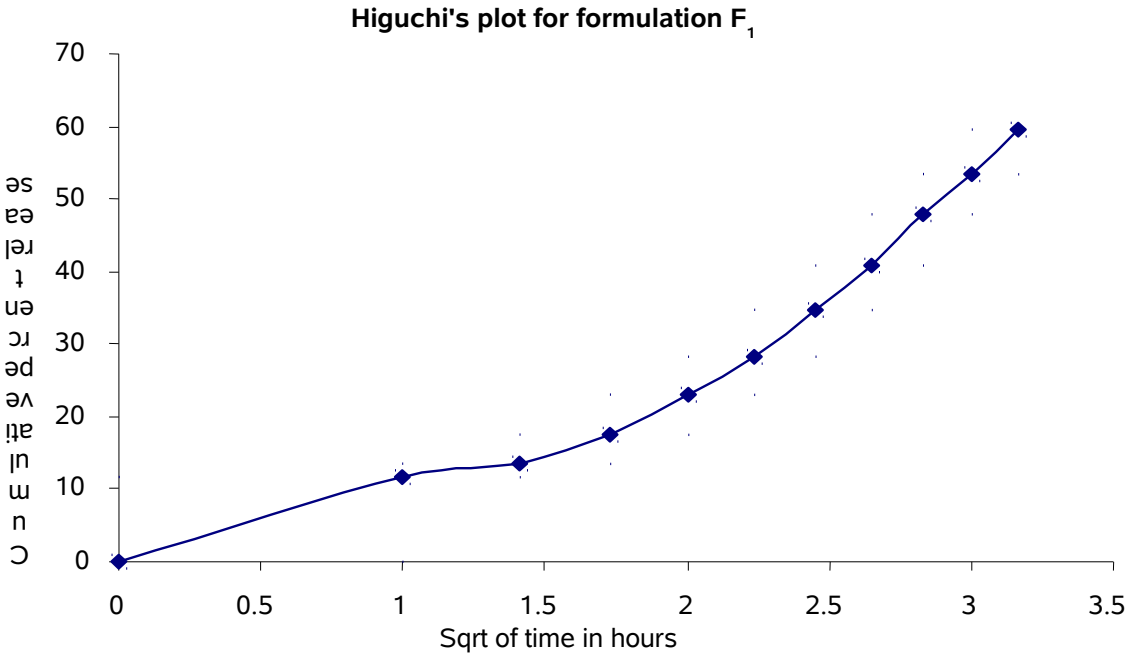
***In vitro* release profile of formulation F_1**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.034	1.10	0.22	11.04	11.60	1.064
2	1.41	0.301	0.041	1.33	0.27	13.31	13.59	1.133
3	1.73	0.477	0.053	1.72	0.34	17.21	17.54	1.244
4	2.00	0.602	0.070	2.27	0.45	22.73	23.16	1.365
5	2.24	0.699	0.085	2.76	0.55	27.60	28.17	1.450
6	2.45	0.778	0.105	3.41	0.68	34.09	34.78	1.541
7	2.65	0.845	0.123	3.99	0.80	39.94	40.79	1.611
8	2.83	0.903	0.144	4.68	0.94	46.75	47.75	1.679
9	3.00	0.954	0.161	5.23	1.05	52.27	53.44	1.728
10	3.16	1.000	0.179	5.81	1.16	58.12	59.42	1.774

Graph No.4



Graph no.5



Graph no.6

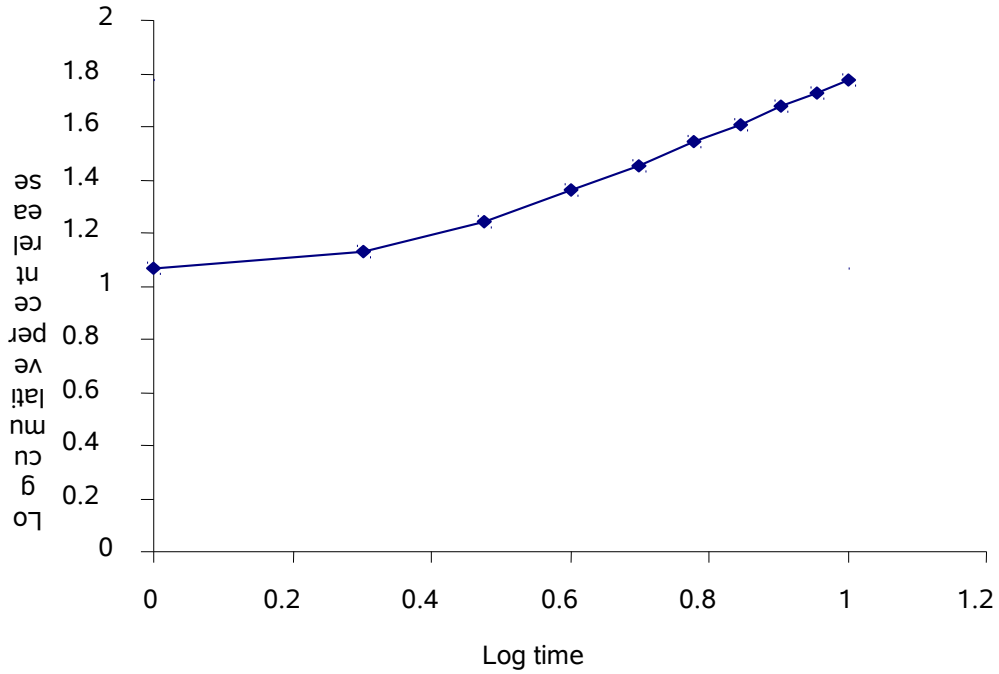
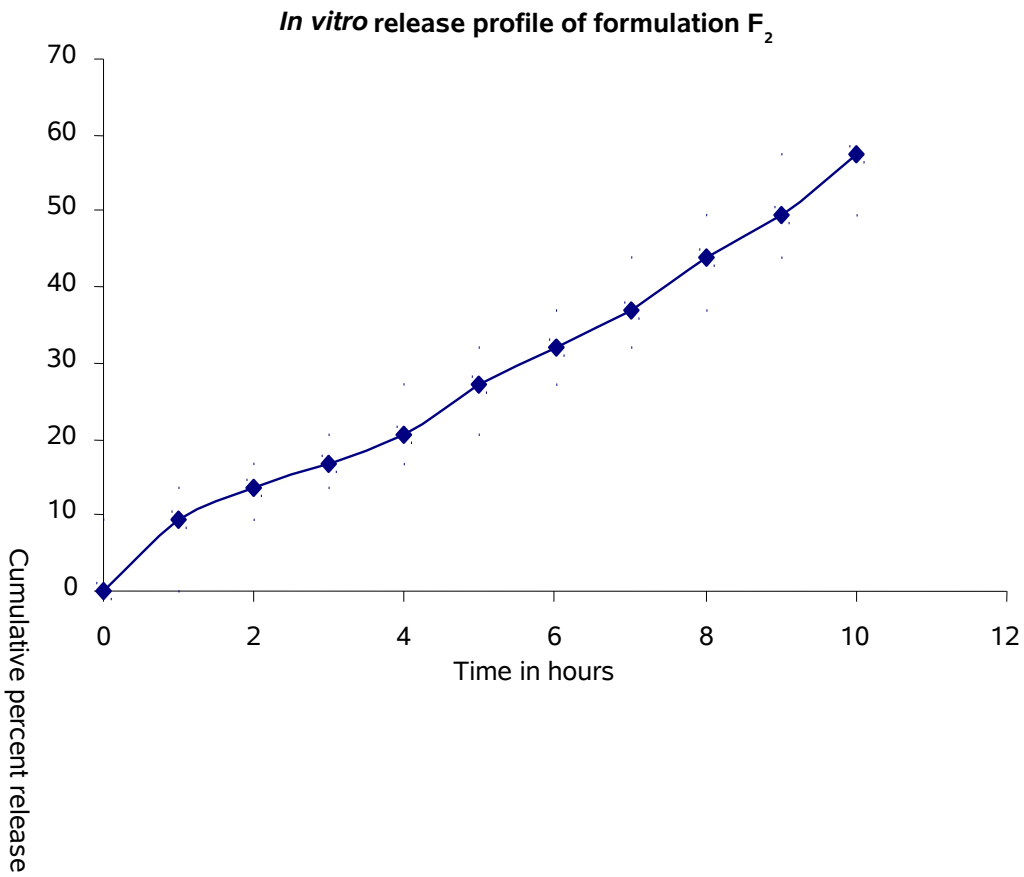


Table no.10

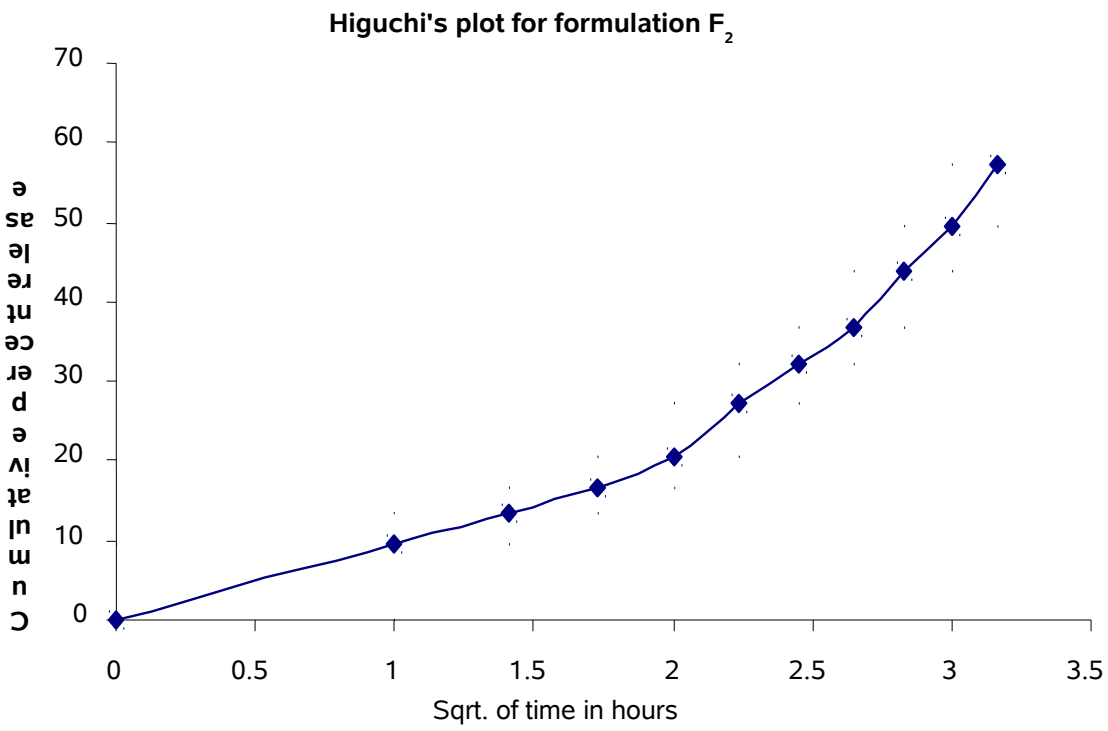
***In vitro* release profile of formulation F_2**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.028	0.91	0.18	9.09	9.56	0.980
2	1.41	0.301	0.041	1.33	0.27	13.31	13.54	1.132
3	1.73	0.477	0.050	1.62	0.32	16.23	16.57	1.219
4	2.00	0.602	0.062	2.01	0.40	20.13	20.54	1.313
5	2.24	0.699	0.082	2.66	0.53	26.62	27.13	1.433
6	2.45	0.778	0.097	3.15	0.63	31.49	32.16	1.507
7	2.65	0.845	0.111	3.60	0.72	36.04	36.83	1.566
8	2.83	0.903	0.132	4.29	0.86	42.86	43.76	1.641
9	3.00	0.954	0.149	4.84	0.97	48.38	49.45	1.694
10	3.16	1.000	0.173	5.62	1.12	56.17	57.38	1.759

Graph no.7


In vitro release profile of formulation F_2
70
60
50
40
30
20
10
0
Cumulative percent release
0
2
4
6
8
10
12
Time in hours

Graph no.8


Higuchi's plot for formulation F2
Cumulative percentage release
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
Sqrt. of time in hours

Graph no.9

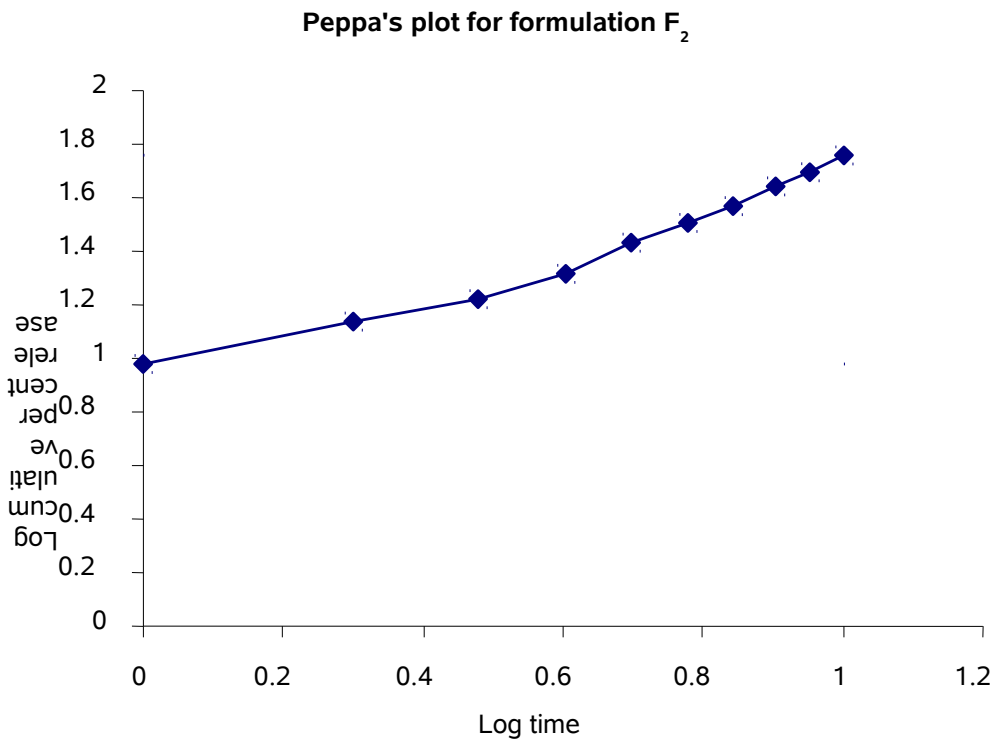
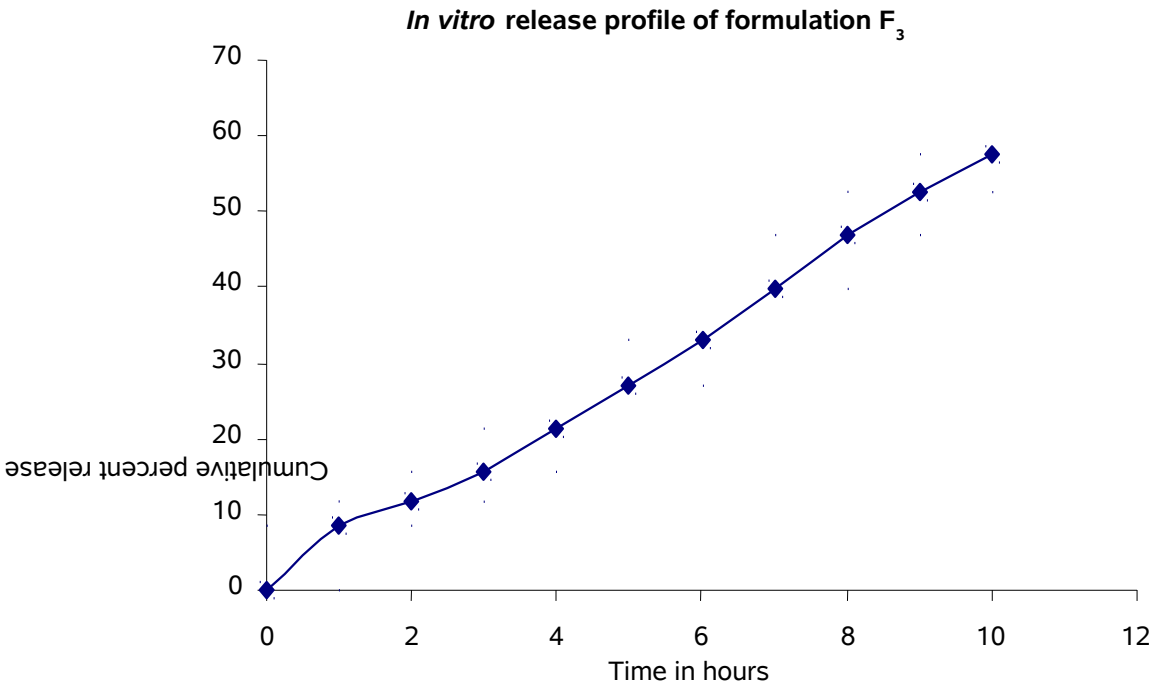

Peppa's plot for formulation F2
Log cumulative percent release
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
Log time

Table no.11

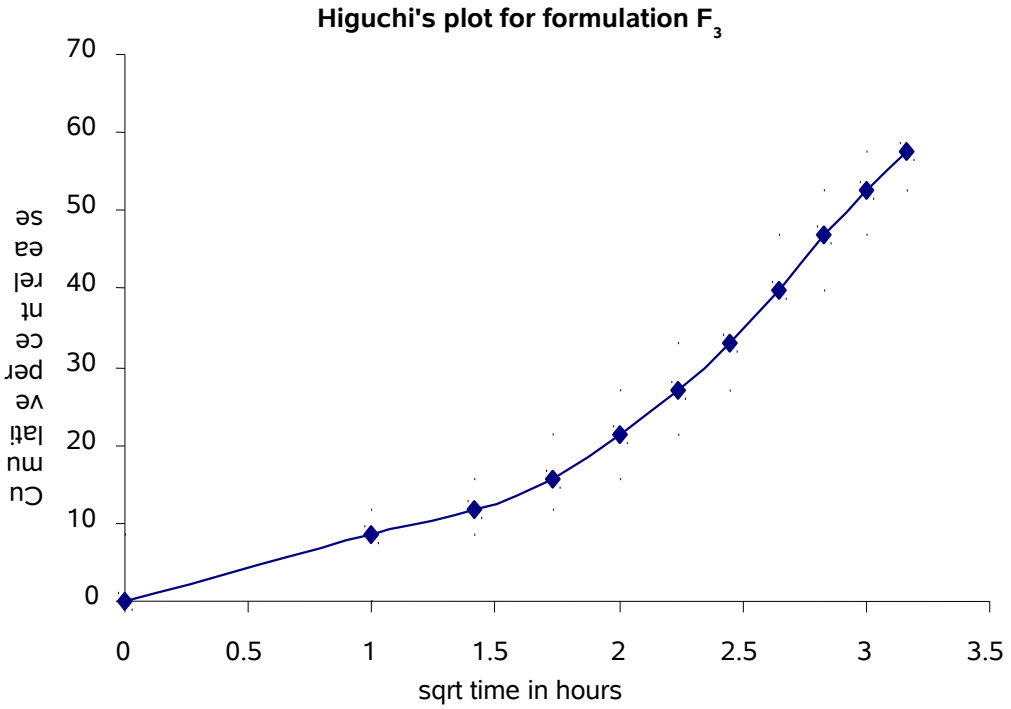
***In vitro* release profile of formulation F_3**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1	0.000	0.025	0.81	0.16	8.12	8.53	0.931
2	1.41	0.301	0.035	1.14	0.23	11.36	11.57	1.063
3	1.73	0.477	0.047	1.53	0.31	15.26	15.54	1.192
4	2	0.602	0.064	2.08	0.42	20.78	21.16	1.326
5	2.24	0.699	0.082	2.66	0.53	26.62	27.14	1.434
6	2.45	0.778	0.100	3.25	0.65	32.47	33.13	1.520
7	2.65	0.845	0.120	3.90	0.78	38.96	39.77	1.600
8	2.83	0.903	0.141	4.58	0.92	45.78	46.75	1.670
9	3	0.954	0.158	5.13	1.03	51.30	52.44	1.720
10	3.16	1.000	0.173	5.62	1.12	56.17	57.45	1.759

Graph no.10



Graph no.11



Graph no.12

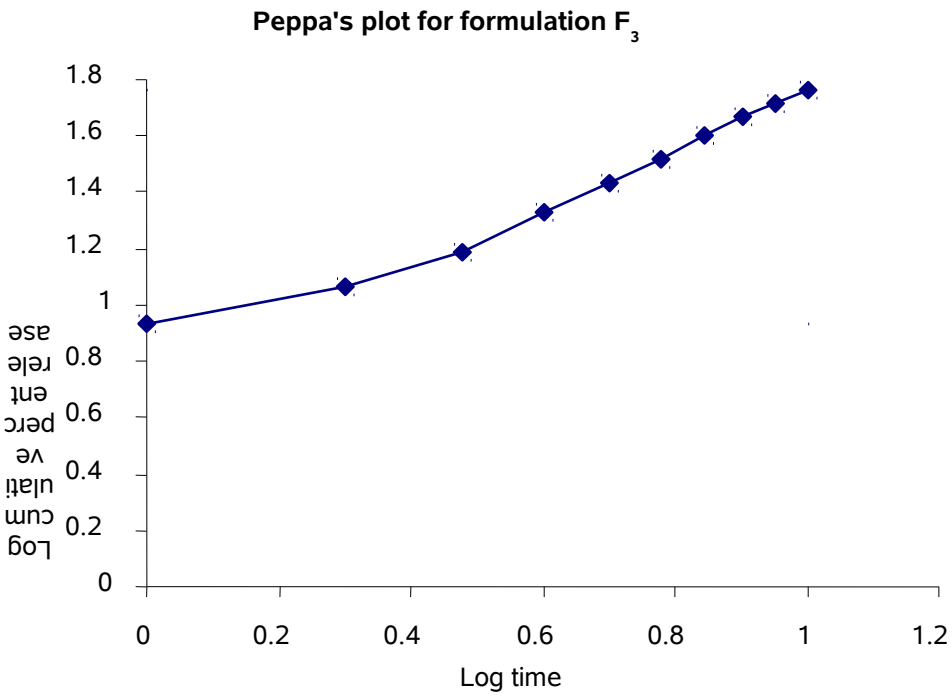


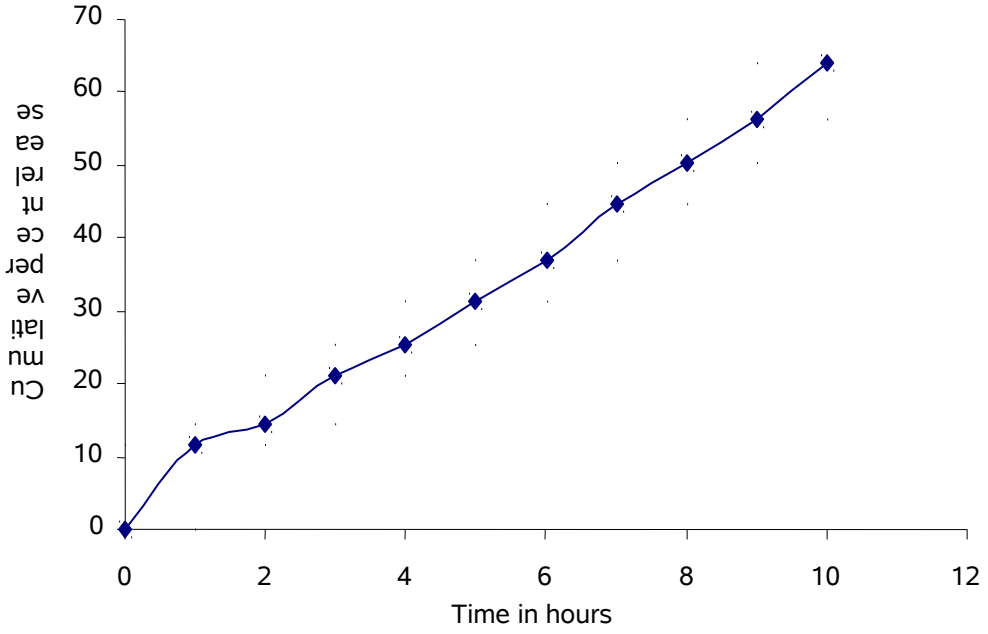
Table no.12

***In vitro* release profile of formulation F_4**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.034	1.10	0.22	11.04	11.60	1.064
2	1.41	0.301	0.044	1.43	0.29	14.29	14.56	1.163
3	1.73	0.477	0.064	2.08	0.42	20.78	21.14	1.325
4	2.00	0.602	0.076	2.47	0.49	24.68	25.19	1.401
5	2.24	0.699	0.094	3.05	0.61	30.52	31.14	1.493
6	2.45	0.778	0.111	3.60	0.72	36.04	36.80	1.566
7	2.65	0.845	0.135	4.38	0.88	43.83	44.73	1.651
8	2.83	0.903	0.152	4.94	0.99	49.35	50.45	1.703
9	3.00	0.954	0.170	5.52	1.10	55.19	56.43	1.751
10	3.16	1.000	0.193	6.27	1.25	62.66	64.04	1.806

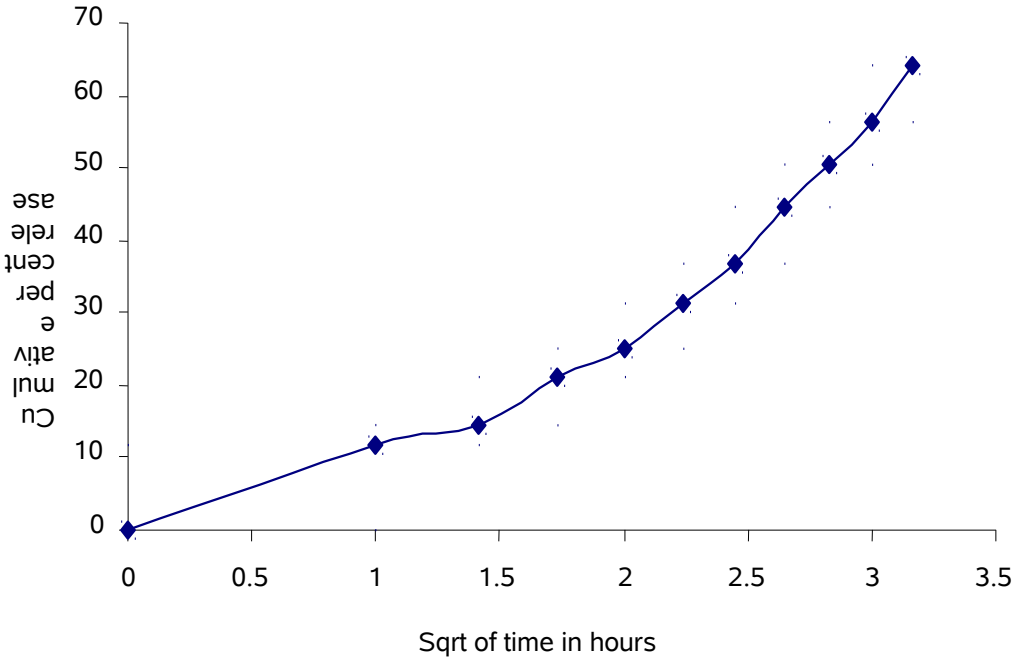
Graph no.13

In vitro release profile of formulation F_4



Graph no.14

Higuchi's plot for formulation F_4



Graph no.15

Peppa's plot for formulation F_4

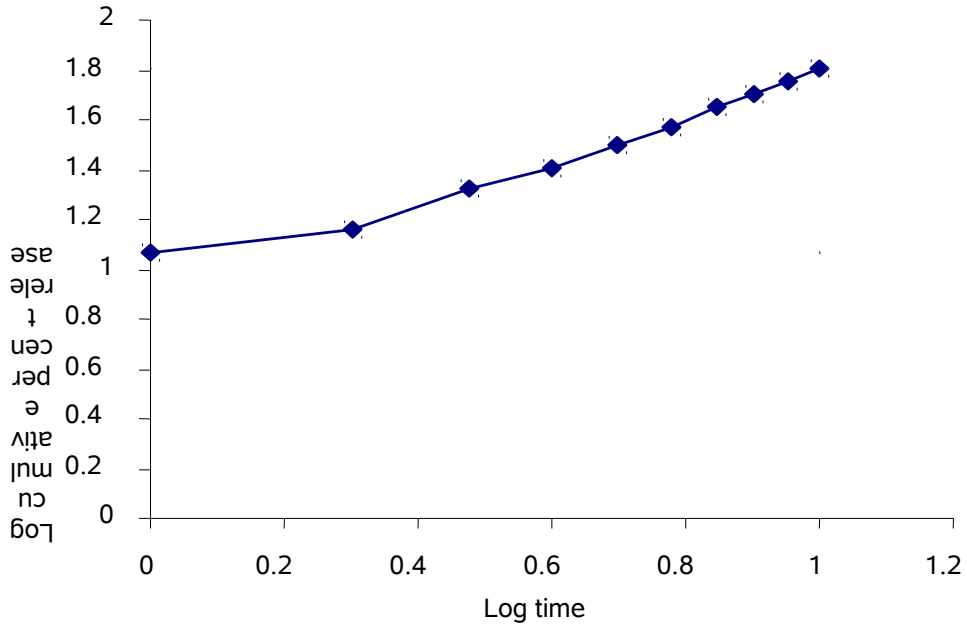
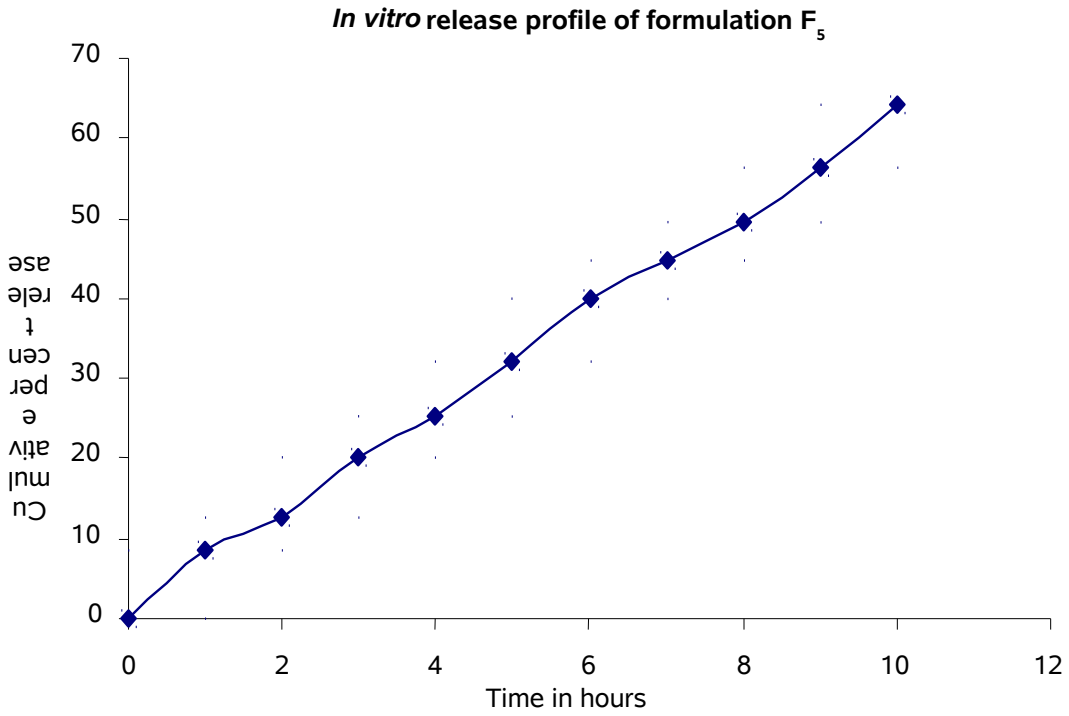


Table no.13

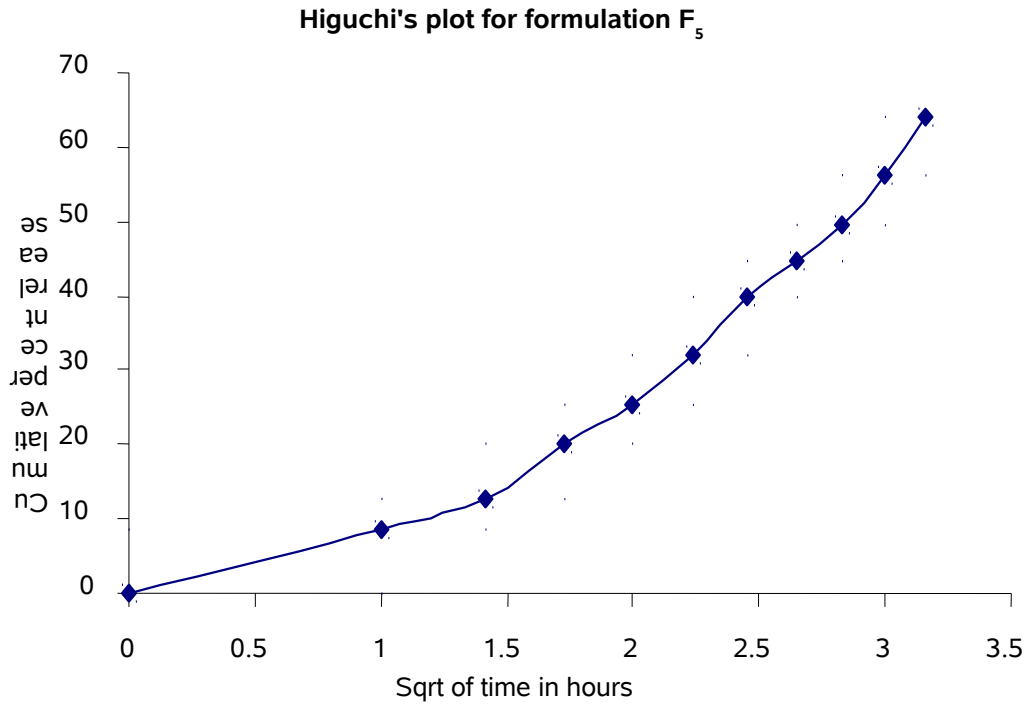
In vitro **release profile of formulation F_5**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.025	0.80	0.16	8.00	8.41	0.925
2	1.41	0.301	0.038	1.24	0.25	12.37	12.57	1.099
3	1.73	0.477	0.062	2.00	0.40	19.98	20.29	1.307
4	2.00	0.602	0.076	2.47	0.49	24.73	25.23	1.402
5	2.24	0.699	0.097	3.14	0.63	31.39	32.01	1.505
6	2.45	0.778	0.120	3.90	0.78	39.00	39.79	1.600
7	2.65	0.845	0.135	4.38	0.88	43.76	44.73	1.651
8	2.83	0.903	0.149	4.85	0.97	48.52	49.61	1.696
9	3.00	0.954	0.170	5.52	1.10	55.18	56.39	1.751
10	3.16	1.000	0.193	6.28	1.26	62.79	64.17	1.807

Graph no.16



Graph no. 17


Higuchi's plot for formulation F5
Cu mu lati ve per ce nt rel ea se
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
Sqrt of time in hours

Graph no. 18

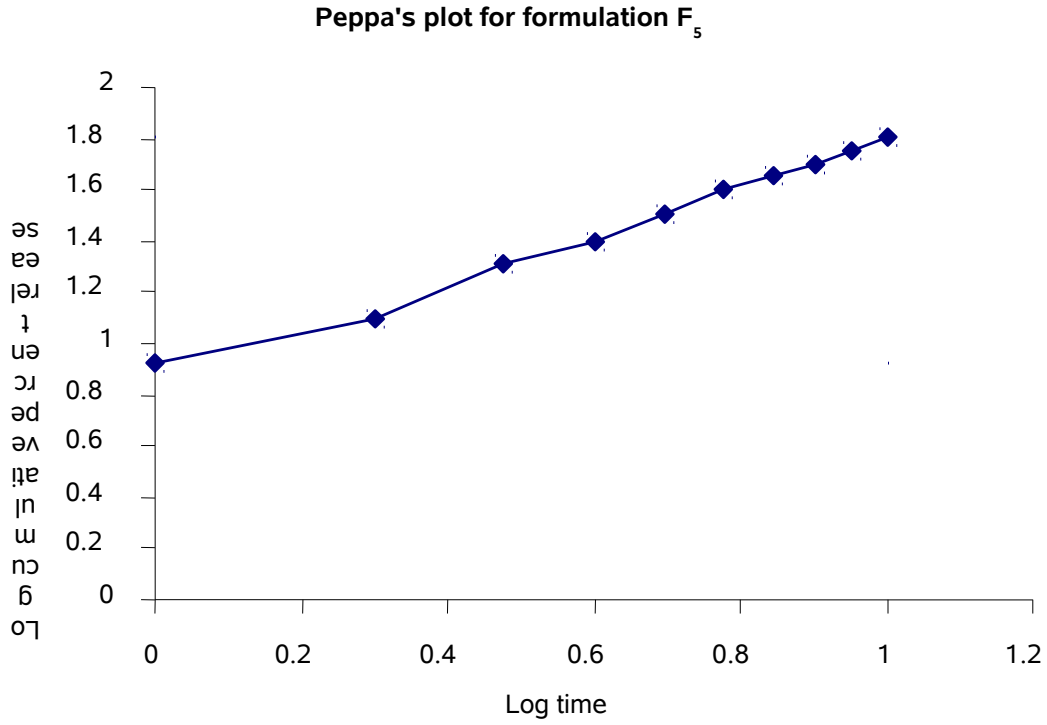
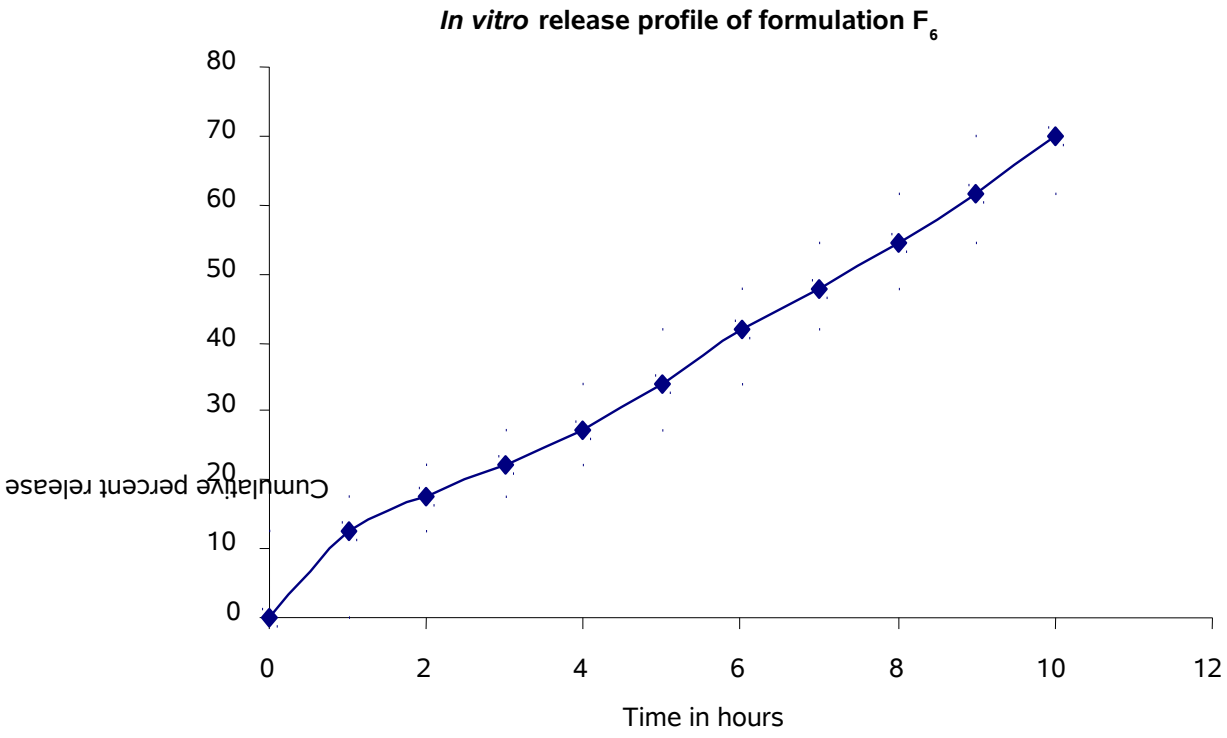

Peppa's plot for formulation F5
Lo g cu m ul ati ve pe rc en t rel ea se
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
Log time

Table no.14

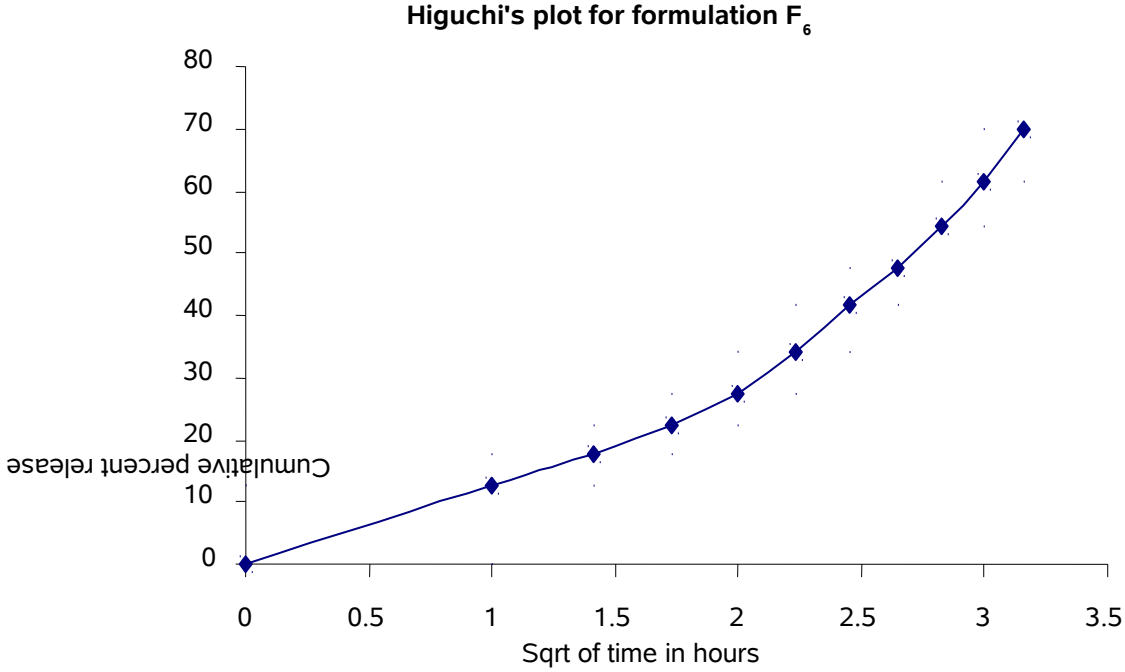
***In vitro* release profile of formulation F_6**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.037	1.20	0.24	12.01	12.63	1.101
2	1.41	0.301	0.053	1.72	0.34	17.21	17.51	1.243
3	1.73	0.477	0.067	2.18	0.44	21.75	22.18	1.346
4	2.00	0.602	0.082	2.66	0.53	26.62	27.17	1.434
5	2.24	0.699	0.103	3.34	0.67	33.44	34.11	1.533
6	2.45	0.778	0.126	4.09	0.82	40.91	41.75	1.621
7	2.65	0.845	0.144	4.68	0.94	46.75	47.78	1.679
8	2.83	0.903	0.164	5.32	1.06	53.25	54.42	1.736
9	3.00	0.954	0.185	6.01	1.20	60.06	61.40	1.788
10	3.16	1.000	0.211	6.85	1.37	68.51	70.01	1.845

Graph no.19



Graph no.20



Graph no. 21

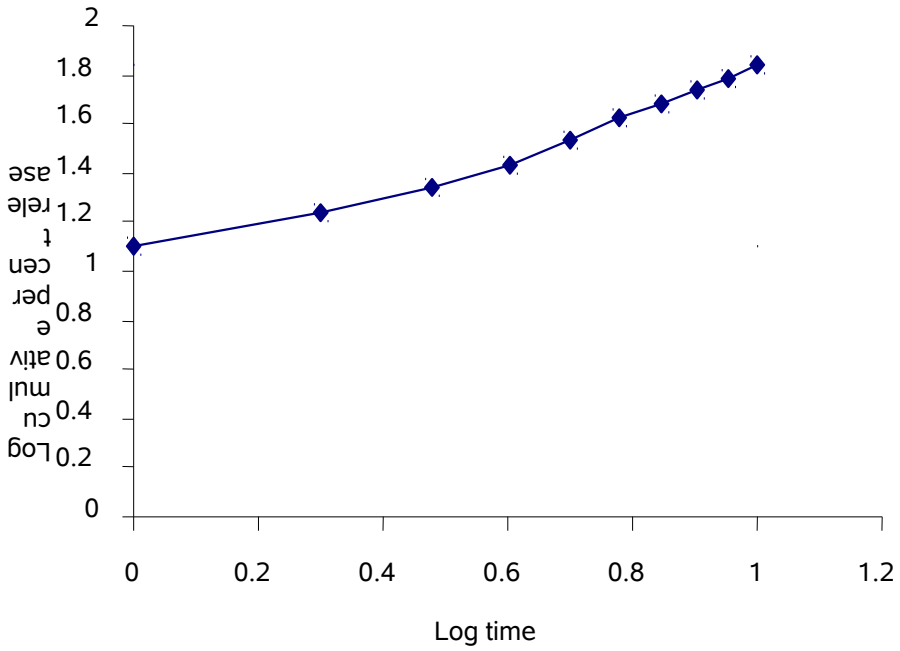
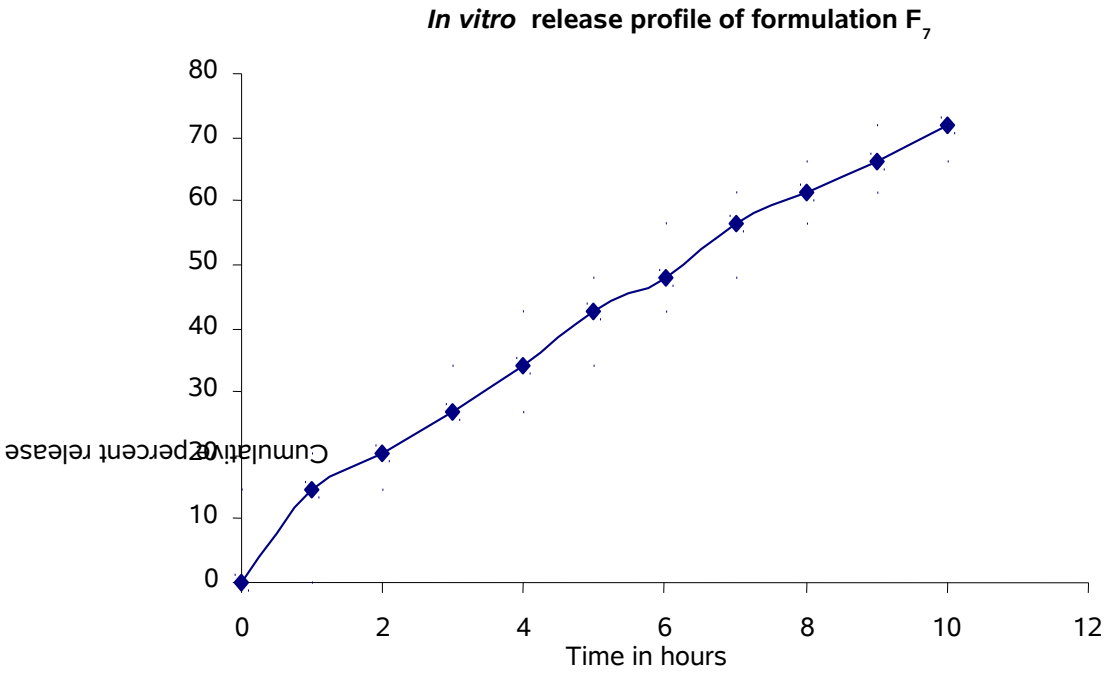


Table no.15

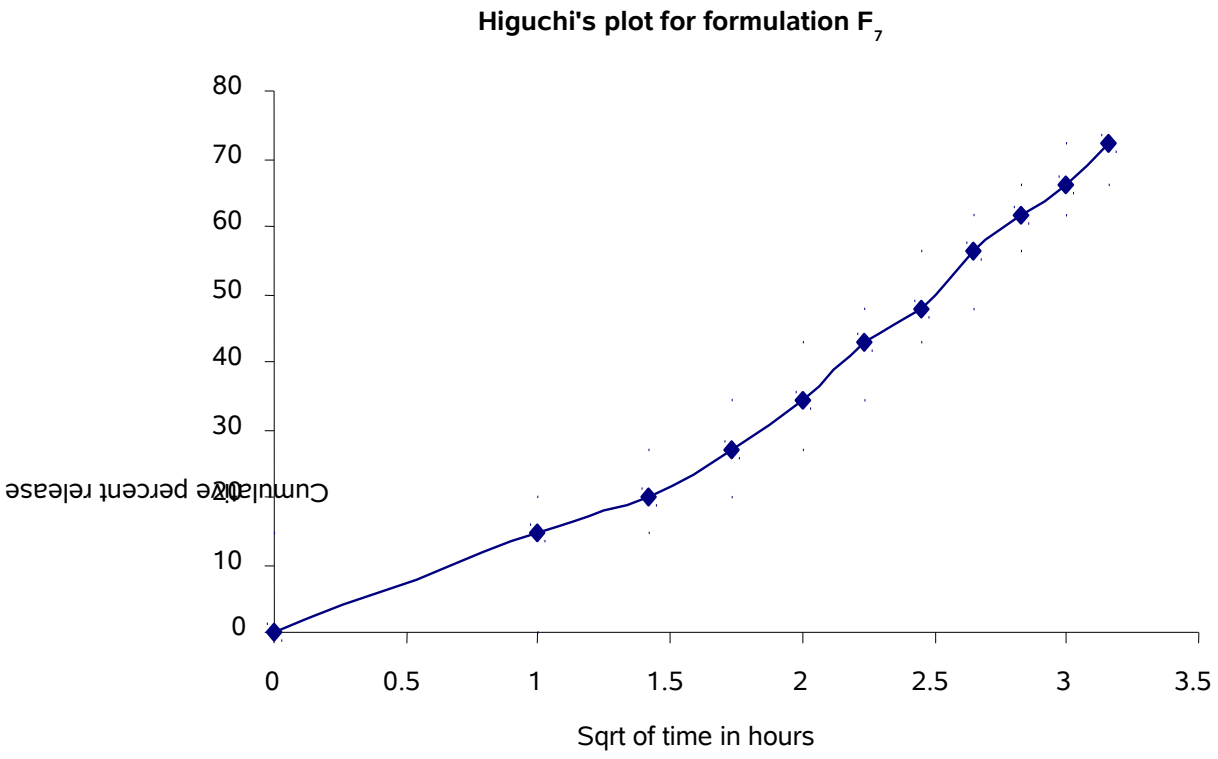
***In vitro* release profile of formulation F_7**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.043	1.40	0.28	13.96	14.72	1.168
2	1.41	0.301	0.061	1.98	0.40	19.81	20.15	1.304
3	1.73	0.477	0.081	2.63	0.53	26.30	26.79	1.428
4	2.00	0.602	0.103	3.34	0.67	33.44	34.10	1.533
5	2.24	0.699	0.129	4.19	0.84	41.88	42.72	1.631
6	2.45	0.778	0.144	4.68	0.94	46.75	47.80	1.679
7	2.65	0.845	0.170	5.52	1.10	55.19	56.36	1.751
8	2.83	0.903	0.185	6.01	1.20	60.06	61.44	1.788
9	3.00	0.954	0.199	6.46	1.29	64.61	66.11	1.820
10	3.16	1.000	0.217	7.05	1.41	70.45	72.07	1.858

Graph no .22



Graph no. 23



Graph no. 24

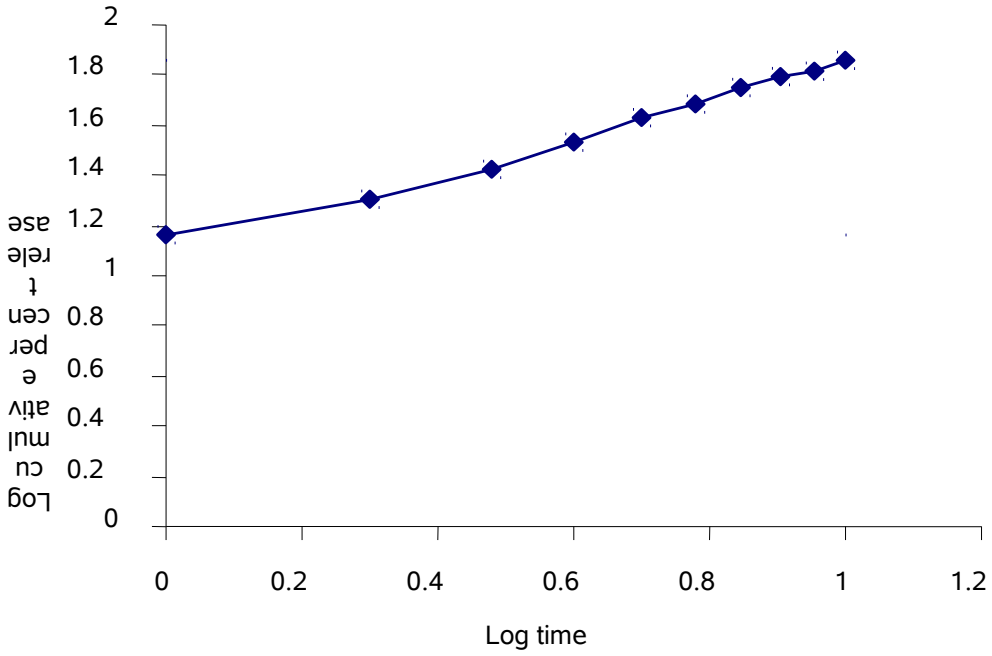


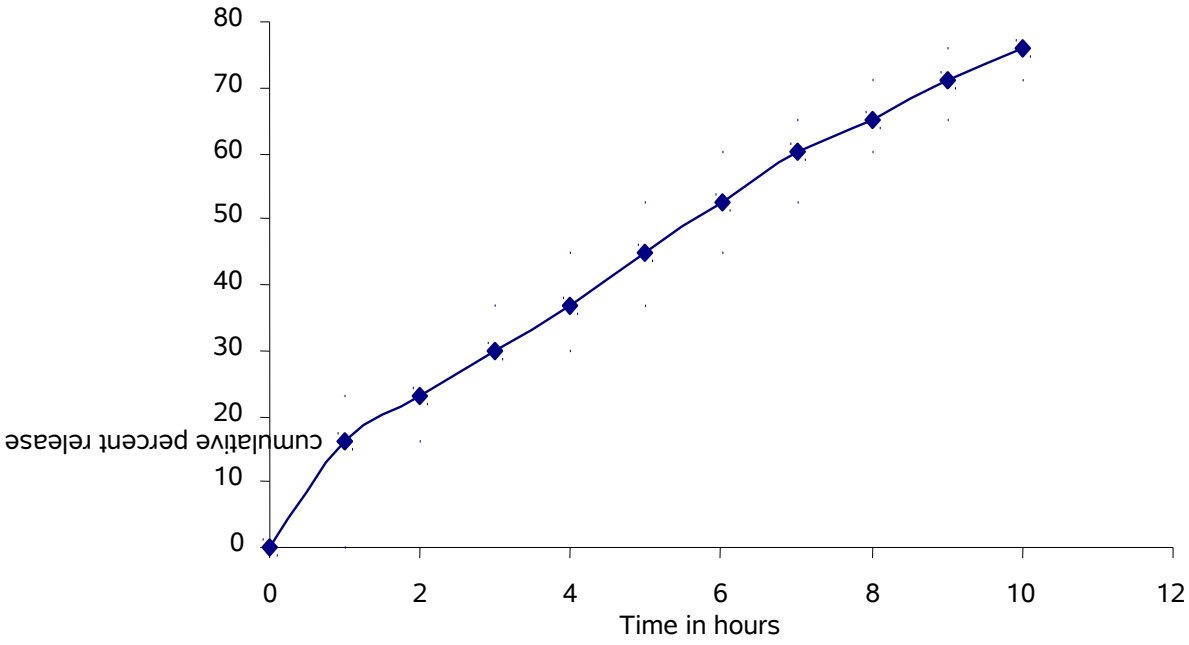
Table no.16

***In vitro* release profile of formulation F_8**

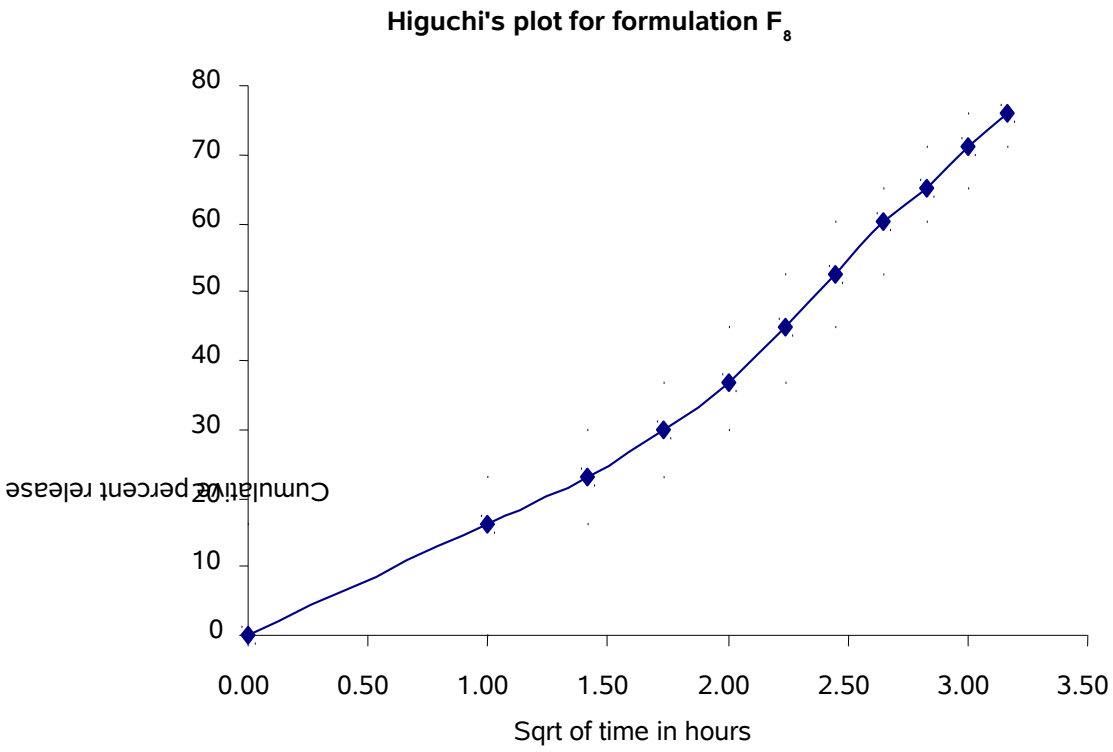
Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.049	1.600	0.320	16.00	16	1.204
2	1.41	0.301	0.070	2.283	0.457	22.83	23.23	1.366
3	1.73	0.477	0.091	2.949	0.590	29.49	30.06	1.478
4	2.00	0.602	0.111	3.615	0.723	36.15	36.89	1.567
5	2.24	0.699	0.135	4.376	0.875	43.76	44.66	1.650
6	2.45	0.778	0.158	5.137	1.027	51.37	52.46	1.720
7	2.65	0.845	0.182	5.898	1.180	58.98	60.26	1.780
8	2.83	0.903	0.196	6.374	1.275	63.74	65.21	1.814
9	3.00	0.954	0.214	6.944	1.389	69.44	71.04	1.851
10	3.16	1.000	0.229	7.420	1.484	74.20	75.94	1.880

Graph no. 25

***In vitro* release profile of formulation F_8**



Graph no. 26



Graph no. 27

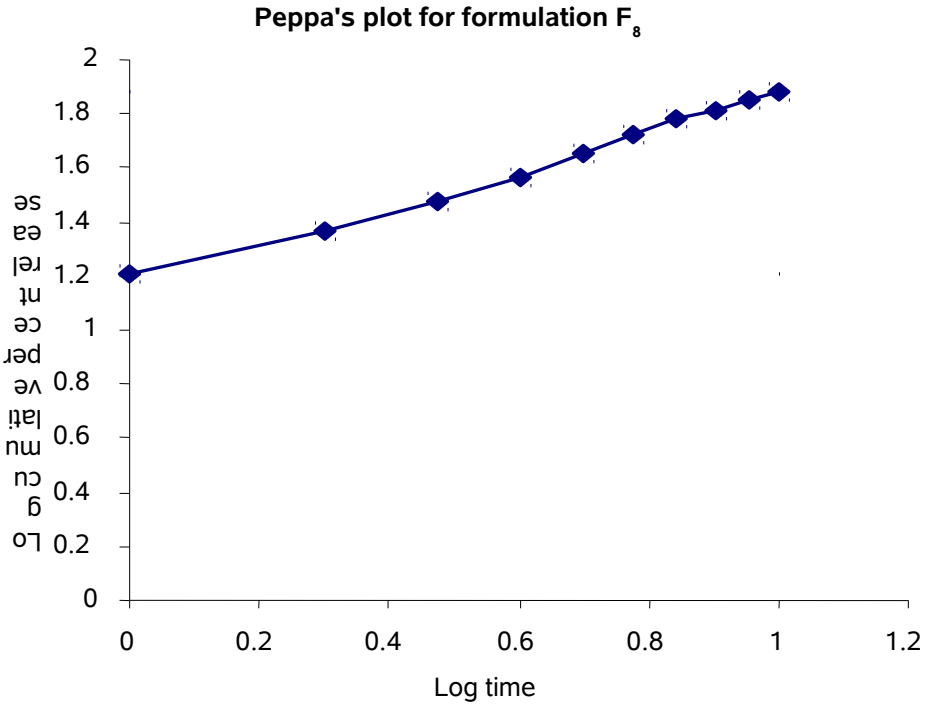
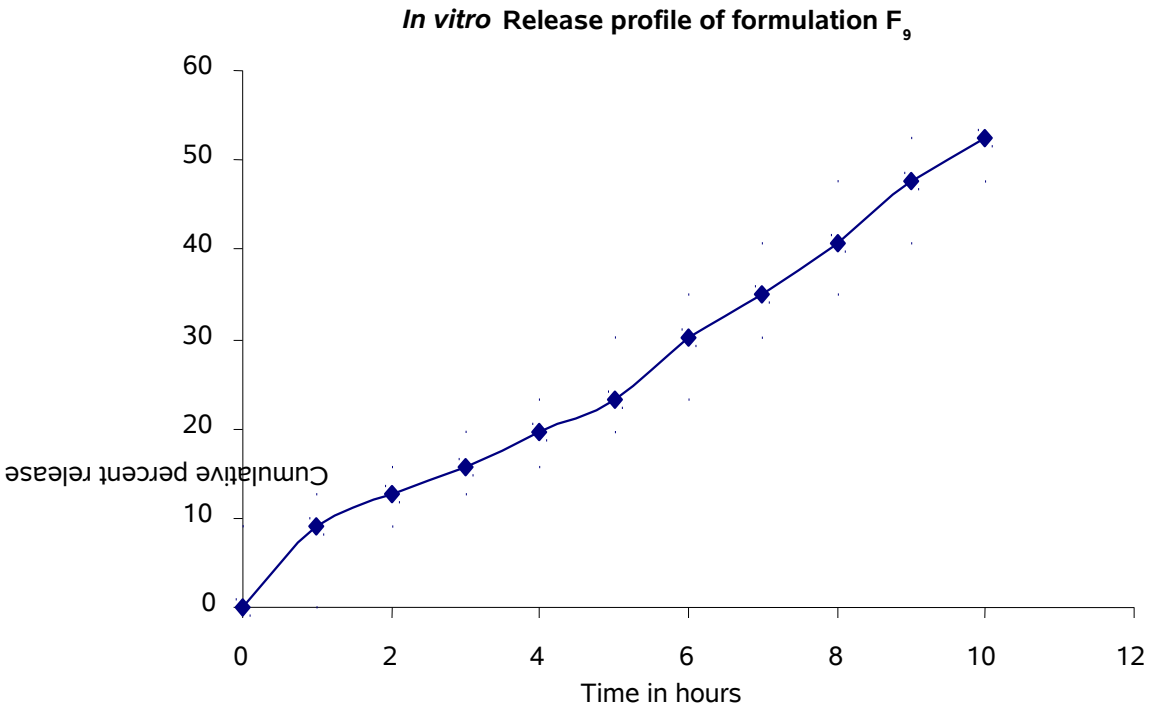


Table no.17

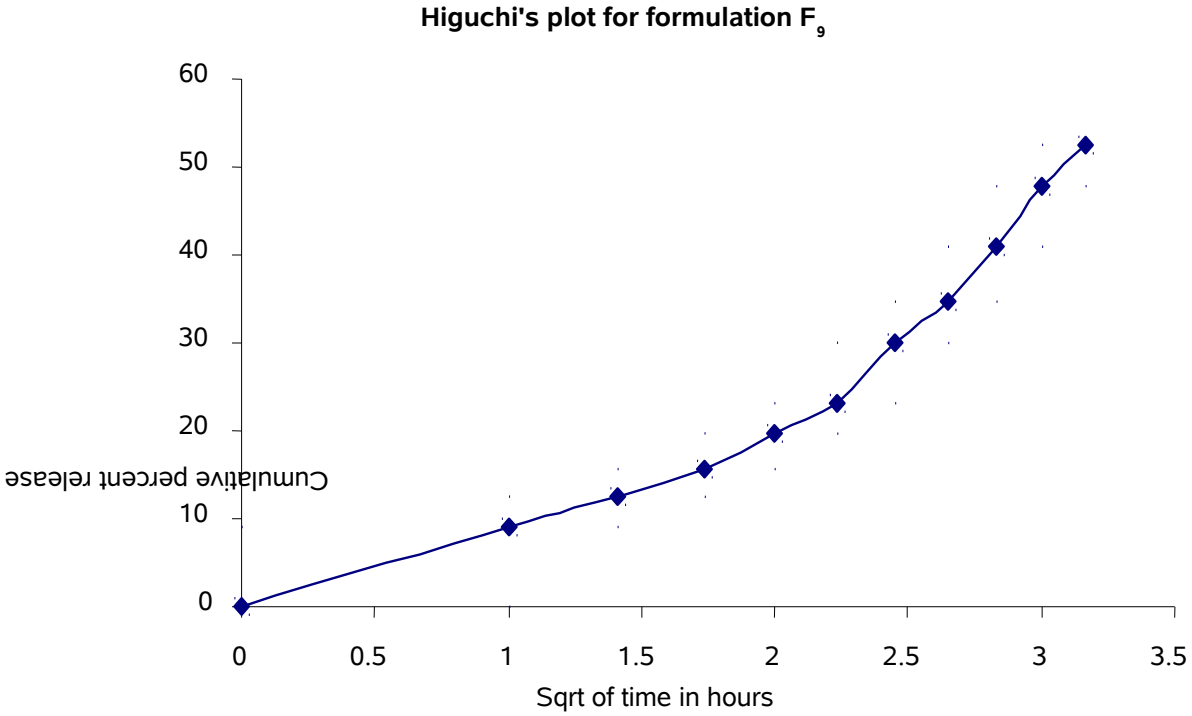
***In vitro* release profile of formulation F_9**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum % release
1	1.00	0.000	0.028	0.909	0.182	9.09	9.09	0.959
2	1.41	0.301	0.038	1.234	0.247	12.34	12.56	1.099
3	1.73	0.477	0.047	1.526	0.305	15.26	15.57	1.192
4	2.00	0.602	0.059	1.916	0.383	19.16	19.54	1.291
5	2.24	0.699	0.070	2.273	0.455	22.73	23.21	1.366
6	2.45	0.778	0.091	2.955	0.591	29.55	30.11	1.479
7	2.65	0.845	0.105	3.409	0.682	34.09	34.83	1.542
8	2.83	0.903	0.123	3.994	0.799	39.94	40.79	1.611
9	3.00	0.954	0.144	4.675	0.935	46.75	47.75	1.679
10	3.16	1.000	0.158	5.130	1.026	51.30	52.47	1.720

Graph no. 28



Graph no. 29



Graph no. 30

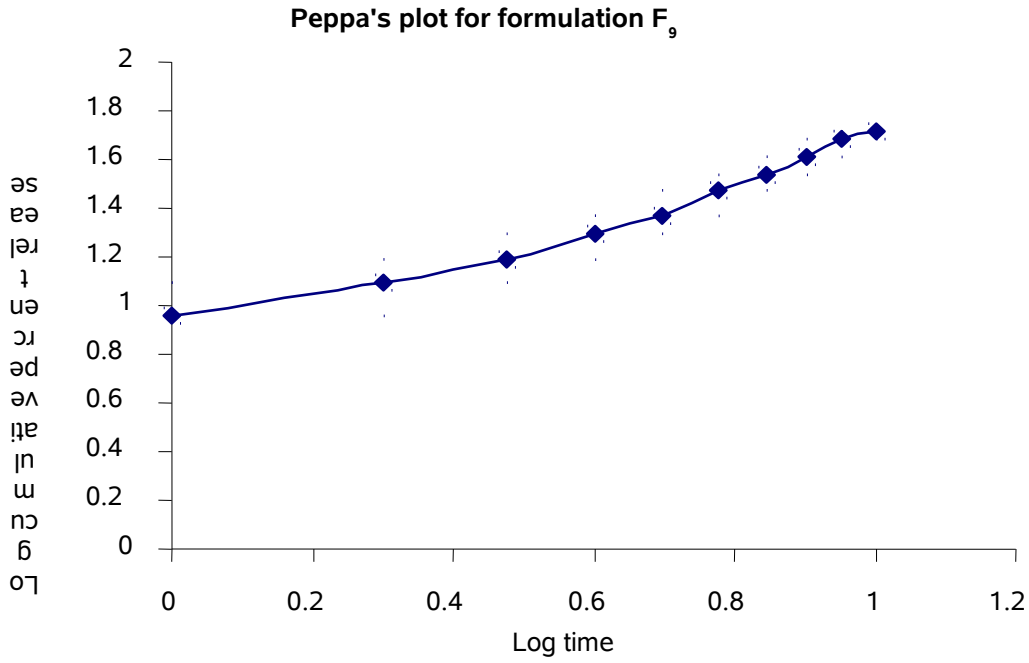
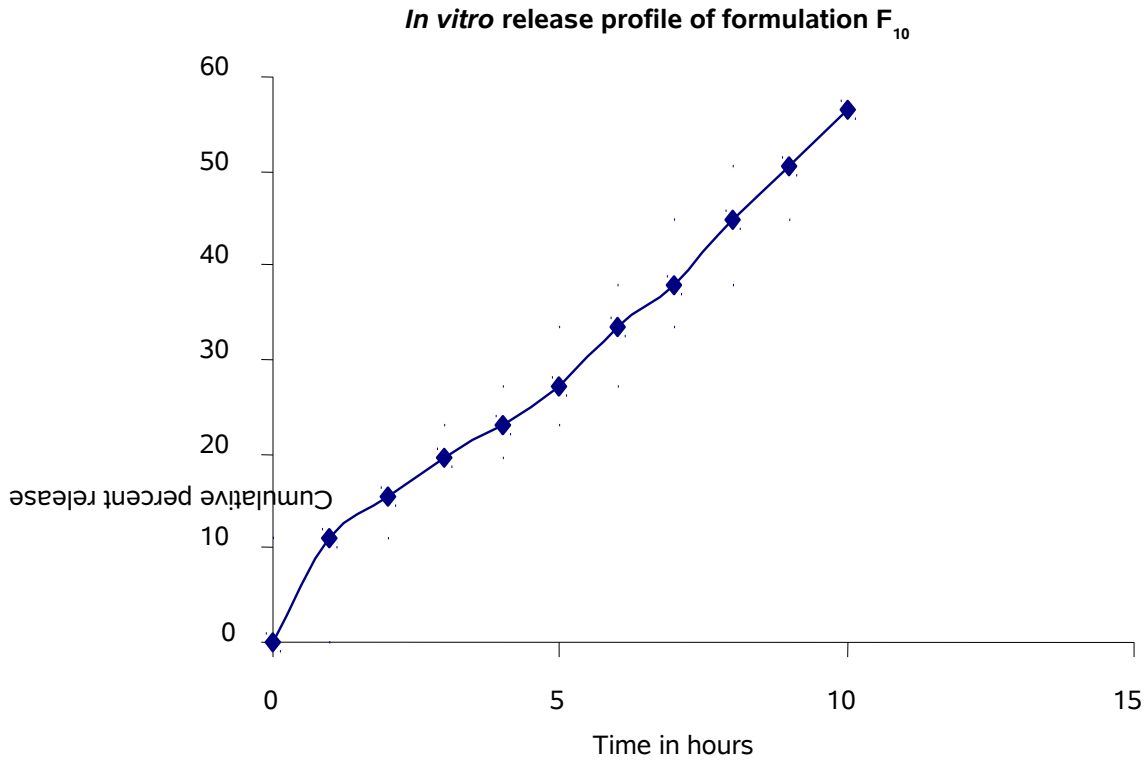


Table no.18

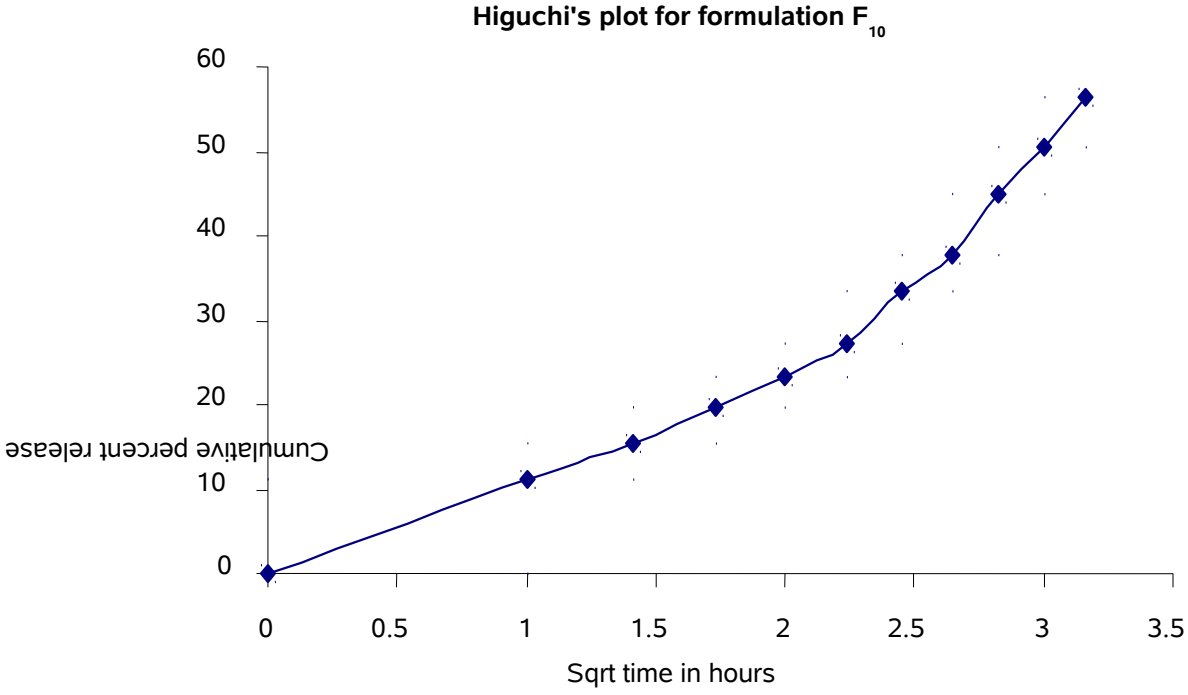
***In vitro* release profile of formulation F_{10}**

Time in hour	Sqrt. of time	Log time	Abs at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.034	1.104	0.221	11.04	11.04	1.043
2	1.41	0.301	0.047	1.526	0.305	15.26	15.54	1.191
3	1.73	0.477	0.059	1.916	0.383	19.16	19.54	1.291
4	2.00	0.602	0.070	2.273	0.455	22.73	23.21	1.366
5	2.24	0.699	0.082	2.662	0.532	26.62	27.19	1.434
6	2.45	0.778	0.101	3.279	0.656	32.79	33.46	1.524
7	2.65	0.845	0.114	3.701	0.740	37.01	37.83	1.578
8	2.83	0.903	0.135	4.383	0.877	43.83	44.76	1.651
9	3.00	0.954	0.152	4.935	0.987	49.35	50.45	1.703
10	3.16	1.000	0.170	5.519	1.104	55.19	56.43	1.751

Graph no. 31



Graph no. 32



Graph no. 33

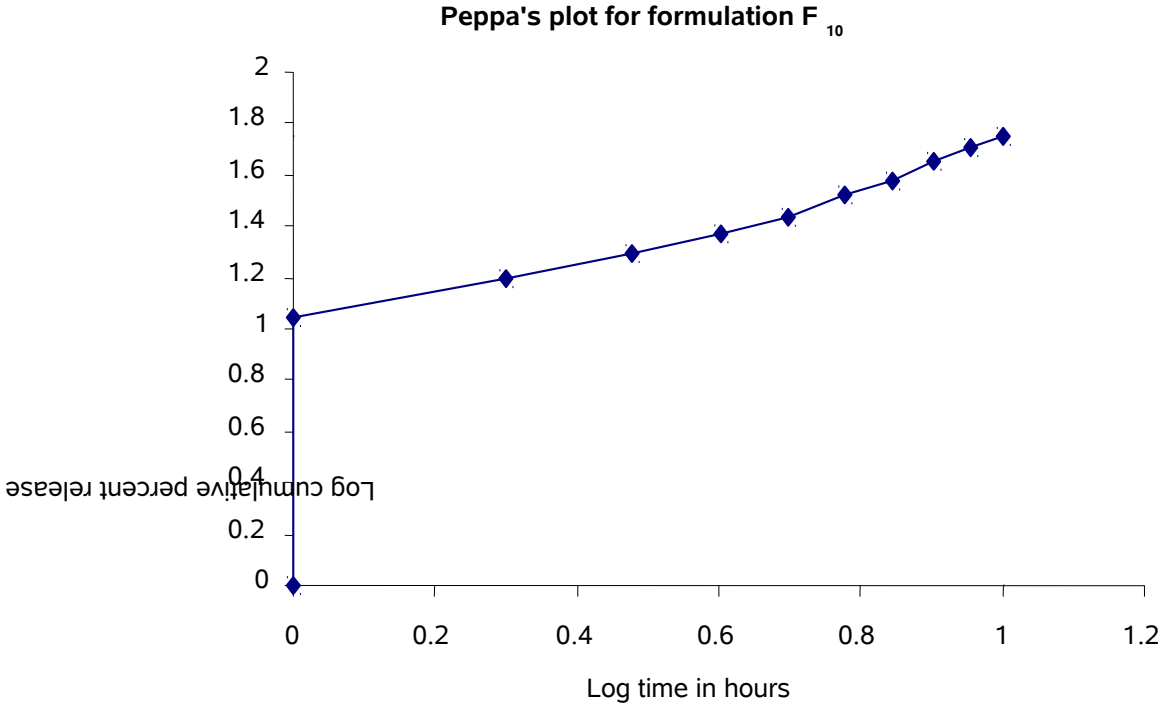


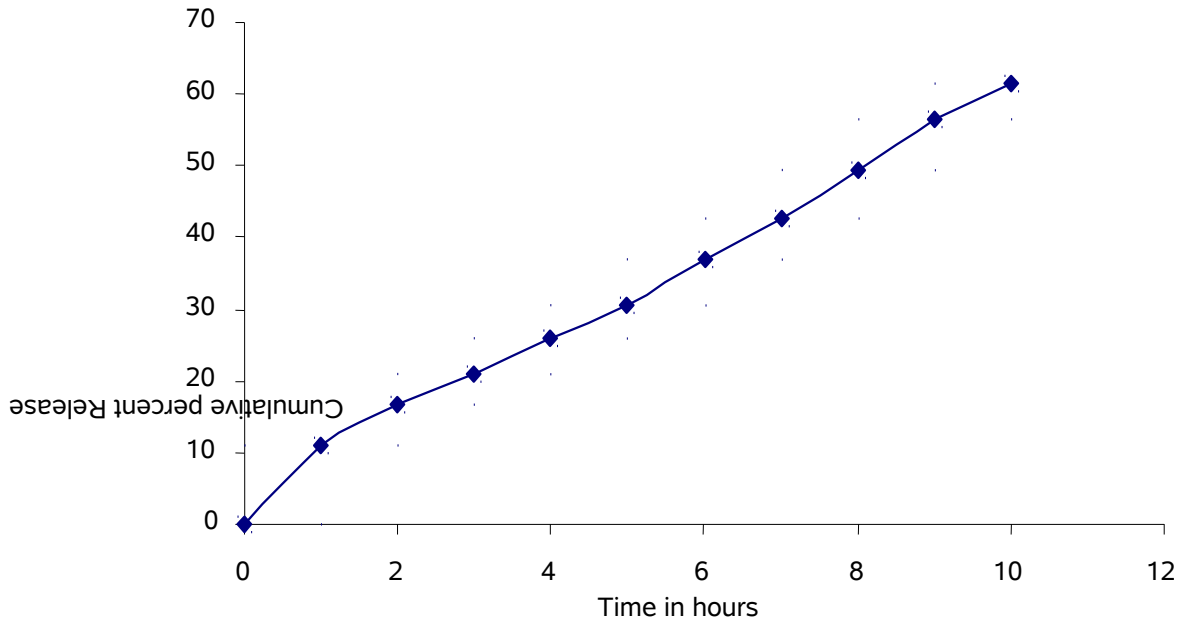
Table no.19

***In vitro* release profile of formulation F_{11}**

Time in hour	Sqrt. of time	Log time	Abs. at 280 nm	Conc. in (µg/ml)	Conc. in 100 ml (Mg/ml)	Percent release	cum% release	Log cum% release
1	1.00	0.000	0.034	1.10	0.221	11.04	11.04	1.043
2	1.41	0.301	0.051	1.66	0.331	16.56	16.83	1.226
3	1.73	0.477	0.063	2.05	0.409	20.45	20.87	1.319
4	2.00	0.602	0.078	2.53	0.506	25.32	25.84	1.412
5	2.24	0.699	0.092	2.99	0.597	29.87	30.50	1.484
6	2.45	0.778	0.111	3.61	0.723	36.15	36.90	1.567
7	2.65	0.845	0.129	4.19	0.837	41.86	42.76	1.631
8	2.83	0.903	0.149	4.85	0.970	48.52	49.56	1.695
9	3.00	0.954	0.170	5.52	1.104	55.18	56.39	1.751
10	3.16	1.000	0.185	5.99	1.199	59.93	61.31	1.788

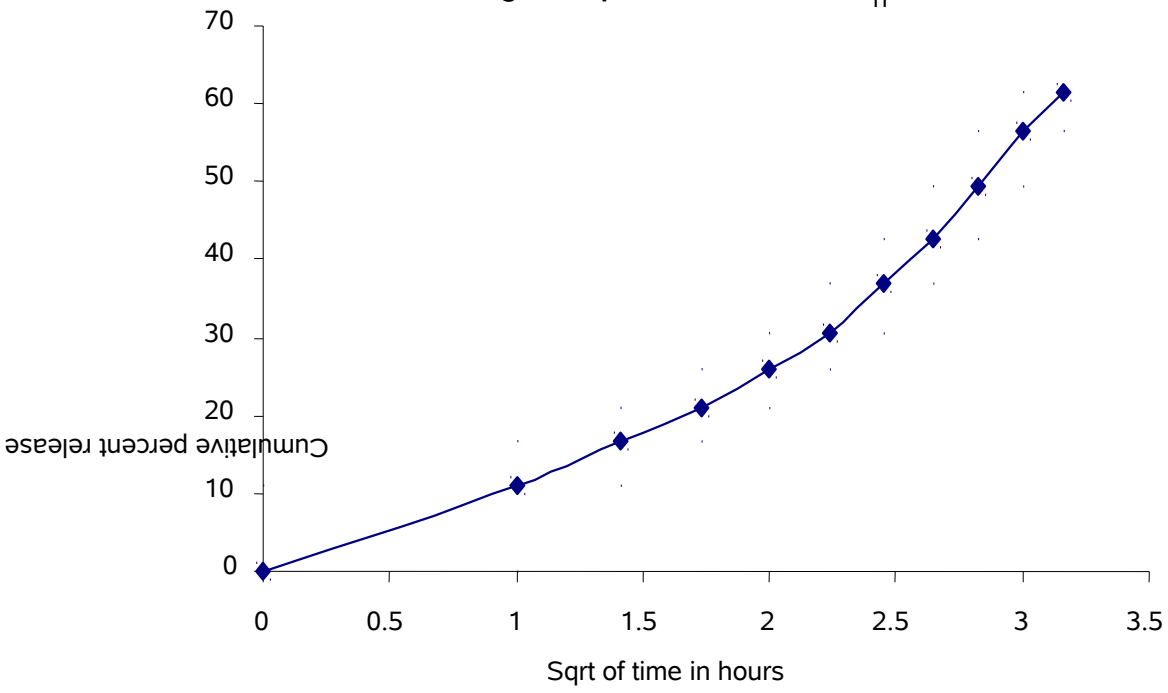
Graph no. 34

***In vitro* release profile of formulation F_{11}**



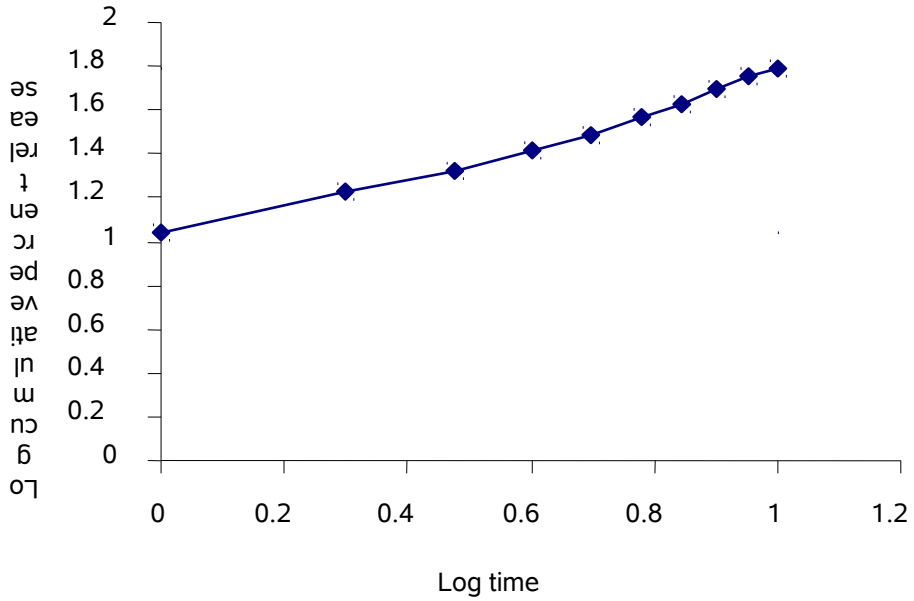
Graph no. 35

Higuchi's plot for formulation F_{11}



Graph no. 36

Peppa's plot for formulation F_{11}



3.3 Drug release kinetic data

The zero order plots showed the zero order release characteristics of the formulation, which was confirmed by the correlation value. In order to find out the mechanism of drug release, the *in vitro* drug release data was graphically treated according to Higuchi's equation and the graphical fit for the *in vitro* data was used to conclude the mechanism of the drug release involved in the delivery system. Correlation value of Higuchi's plot revealed that the mechanism of drug release is diffusion. The correlation value of zero order plots and correlation value of Higuchi's plot presented in table no. 22. The *in vitro* kinetic data subjected to log time log drug release transformation plot (peppa's model), all the value ranges from 1 to 1.2704 revealed the fact that the drug release follows a super case II transport diffusion. The slop value for each formula presented in table below.

Table no. 20

Drug release kinetic data

Formulations	Zero order		Higuchi's		Peppa's	
	Slope	Correlation	Slope	Correlation	Slope	Correlation
F_1	5.6826	0.9956	18.2973	0.9542	1.2040	0.8691
F_2	5.3567	0.9952	17.7922	0.9511	1.2074	0.9823
F_3	5.7173	0.9974	18.8995	0.9487	1.2704	0.9039
F_4	6.0543	0.9970	20.2571	0.9598	1.2267	0.8696
F_5	6.2483	0.9989	28.9314	0.9938	1.0000	0.9040
F_6	6.5599	0.9967	21.9748	0.9607	1.2358	0.8613
F_7	6.9329	0.9943	23.4715	0.9797	1.2392	0.8448
F_8	7.2887	0.9929	25.0848	0.9832	1.2386	0.8341
F_9	5.0068	0.9945	16.6064	0.9491	1.1898	0.8846
F_{10}	5.2206	0.9947	17.5716	0.9633	1.1661	0.8589
F_{11}	5.8270	0.9967	19.6144	0.9653	1.2107	0.8652

4. STABILITY STUDY

4.1 Physical stability

Physical stability was carried out to investigate the leaching out of the diclofenac sodium from niosomes at refrigerated temperature, as shown in the graph no: 2. The percent of diclofenac retained in the span 60 vesicle after a period of three months were 82.47%, 78.32% and 76.43% respectively for formulations F_4 (200:125), F_5 (200:115) and F_6 (200:100). Also the results indicate that more than 80% of diclofenac sodium was

retained in the niosomal formulation for a period of 60 days. From this it can be concluded that vesicles are stable enough to store under refrigeration temperature with least leakage. The leakage of drug from F_6 may be due to its higher surfactant content and lower cholesterol which formed a leaking vesicle.

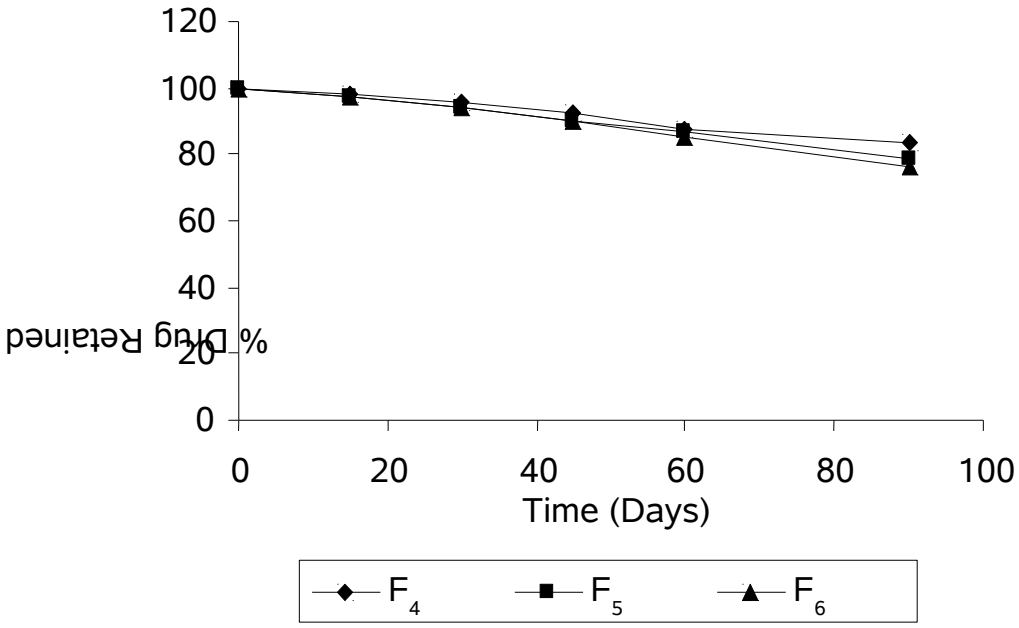
Table No.21

Percentage of diclofenac sodium retained on refrigerated storage

S.No.	Days Stored	F_4 Percent retained	F_5 Percent retained	F_6 Percent retained
1	15	98.35	97.33	97.42
2	30	95.32	94.23	93.75
3	45	92.66	90.26	89.84
4	60	87.22	86.37	84.79
5	90	83.47	78.32	76.43

Graph No. 2

Percentage of diclofenac sodium retained in the niosome formulations after storage at refrigeration temperature



4.2. Test of Significance

The stability data analyzed for significant difference between retention patterns of drug in three different niosomal formulations on storage. The test value showed no significant difference (P>0.05) between the stability data of formulations from each other.

Table No: 22
Test of Significance

Formulations	F4-F5	F5-F6	F4-F6
P-value	0.060484	0.084811	0.050935

4.3. Zeta potential analysis

The formulation F_6 which was subjected to zeta potential analysis had a zeta value of +29mv, which is a measure of net charge of niosomes. This higher charge on the surface of vesicle produce a repulsive force between the vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension. From this it can be concluded that formulation F_6 provides much stable Niosomal suspension.

5. *IN-VIVO* STUDY

The prepared niosomes of diclofenac sodium studied for its prolonged release *in vivo* in the animal model Rabbit (n=2). *In vivo* study conducted to investigate the ocular availability of drug for a prolonged action after a single dose. The study carried out by comparing the retention time of the standard drug solution to that of the aqueous humors extracted sample. The retention time obtained for the standard was 4.52 minutes, for the samples at 4^{th} and 8^{th} hour were 4.780 and 4.660 minutes respectively. This matching retention time of three injections to HPLC showed the presence of drug diclofenac sodium in the aqueous humor sample even after 4^{th} and 8^{th} hour of administration. In other words drug was available in detectable quantities even after 8^{th} hour of administration, where as the literature says, concentration of diclofenac sodium in aqueous humor will be in undetectable quantity after 4^{th} hr of administration as conventional ocular drops. This may be because of possible retention of drug in the aqueous humor due to high corneal contact time and permeability provided by the vesicular system.

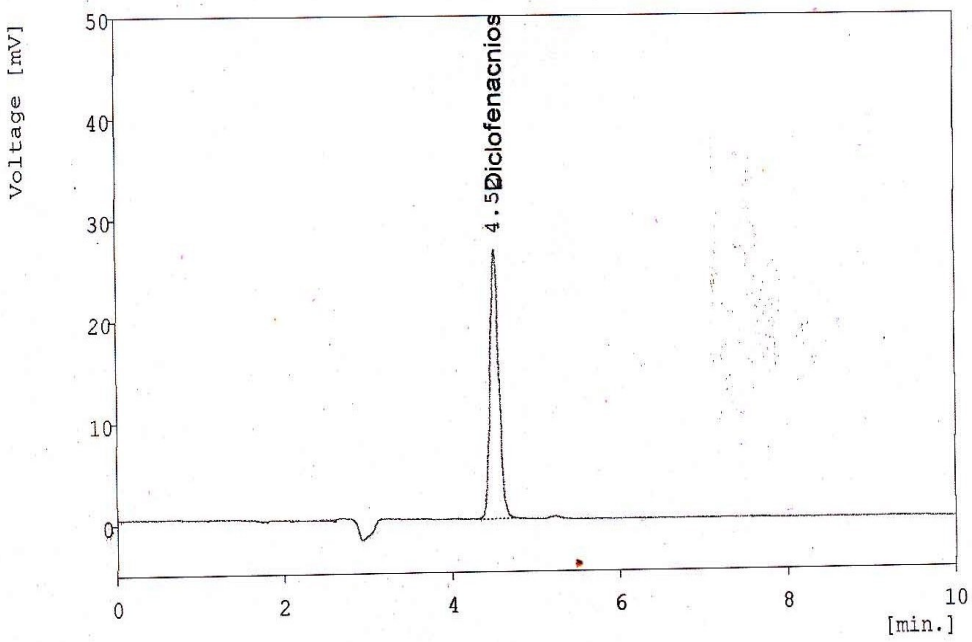
H.P.L.C peak for standard solution

Method : @d50mcg
Diclofenac.neo

Created : Thu, 29th Nov, 2007 12:14:09 By : m.g

Peak Width : 0.3 min. Threshold : 0.1 mV Detect Negative : Disabled
Integration Start : 0 min.

Base : Area Calibration File : (None) Calculation : Uncal
Scale Factor : 1 Units After Scaling : ul Uncal. Response : 0
Dead Time : 1 min. Column Length : 100 mm



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.520	193.4526	26.6205	0.1200	100.0000	100.0000
-	Total	193.4526	26.6205			

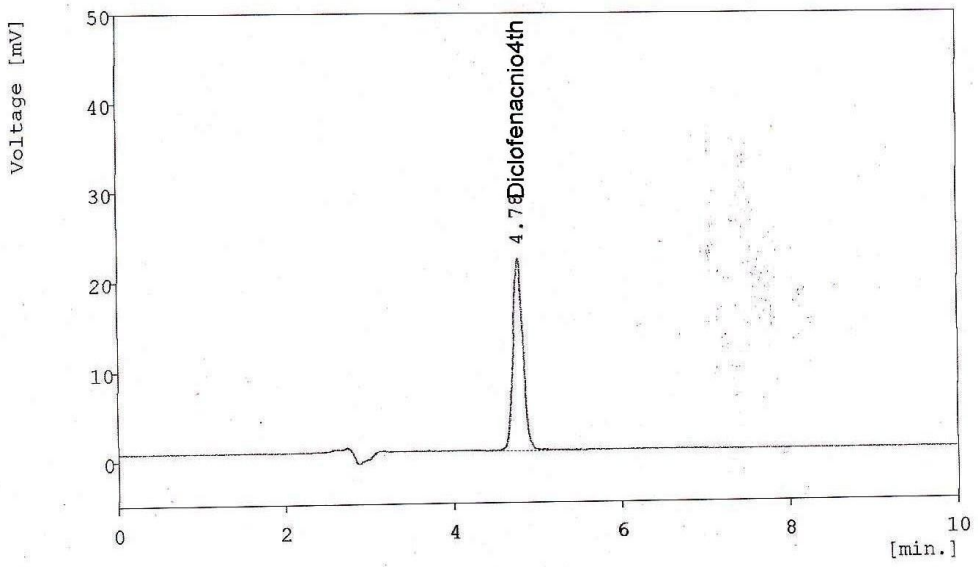
Fig no. 7: Standard HPLC peak of diclofenac sodium with retention time at 4.520

HPLC peak for sample at 4th hour

Method : d4th
Diclofenac.neo
Created : Thu, 29th Nov, 2007 12:14:09 By : m.g

Peak Width : 0.3 min. Threshold : 0.1 mV Detect Negative : Disabled
Integration Start : 0 min.

Base : Area Calibration File : (None) Calculation : Uncal
Scale Factor : 1 Units After Scaling : ul Uncal. Response : 0
Dead Time : 1 min. Column Length : 100 mm



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.780	178.7018	21.5752	0.1300	100.0000	100.0000
-	Total	178.7018	21.5752			

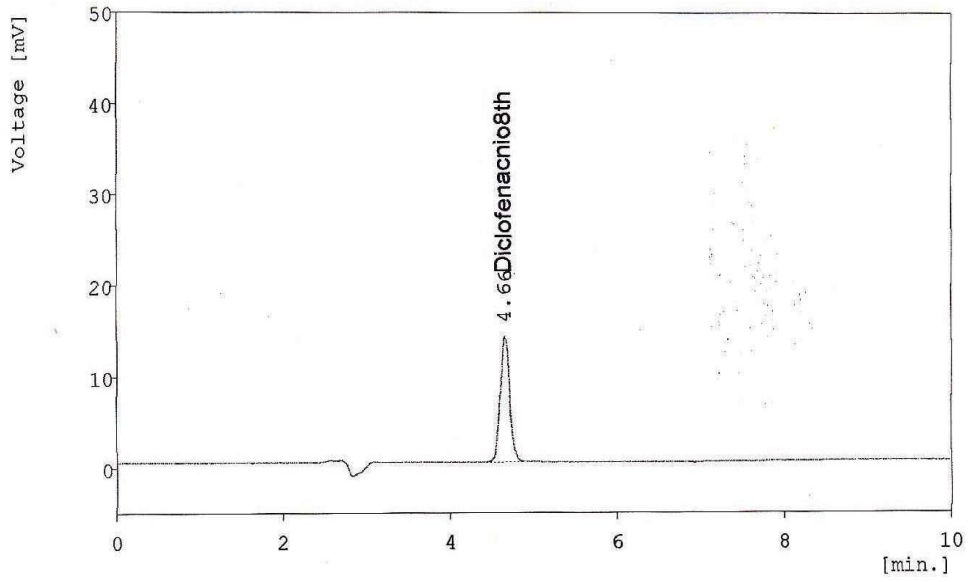
Fig no:8 Sample peak at 4th hour after drug administration having a retention time of 4.780 min.

HPLC peak for sample at 8th hour

Method : d8th
Diclofenac.neo
Created : Thu, 29th Nov, 2007 12:14:09 By : m.g

Peak Width : 0.3 min. Threshold : 0.1 mV Detect Negative : Disabled
Integration Start : 0 min.

Base : Area Calibration File : (None) Calculation : Uncal
Scale Factor : 1 Units After Scaling : ul Uncal. Response : 0
Dead Time : 1 min. Column Length : 100 mm



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.660	108.5448	13.8224	0.1300	100.0000	100.0000
-	Total	108.5448	13.8224			

Fig no : 9 Sample peak at 8th hour after administration of drug having retention time of 4.660 min.

CONCLUSION

Recently niosomes have been studied by many workers as a choice of ocular drug delivery system to provide a better ocular bioavailability considering, high penetration property of the niosome encapsulated agents through biological membrane and the stability of them.

The present formulation study on diclofenac sodium is an attempt to prepare niosomal drug delivery system and evaluate its performance. The formulations were prepared with different ratios of cholesterol and surfactant.

An ideal or best formulation of niosome is said to be one which gives high entrapment efficiency. In this study entrapment efficiency is found to be cholesterol:surfactant ratio dependent. The release rate also found to be dependent of cholesterol:surfactant ratio. The formulation $F_{6,}$ which showed a higher entrapment efficiency provides a comparatively less leaky niosome this fact confirms the above said. Zeta potential study proved that the above formulation have an excellent stability. Formulations also found to ensures a good ocular bioavailability of the drug *in vivo*.

By these facts study can be concluded by saying niosomes formed from span 60 and cholesterol in the ratio 200:100 (in μmol) is a promising approach to improve the bioavailability of diclofenac sodium even for an extended period of time.

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